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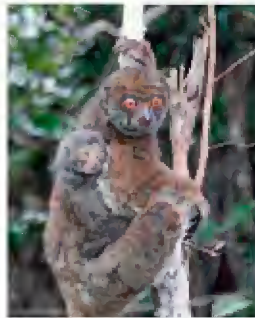
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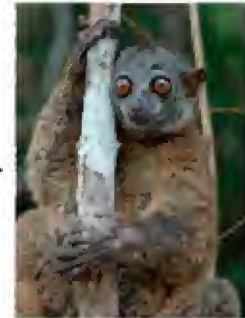
15 February 2008

NOCTURNAL LEMUR DIVERSITY AT MASOALA NATIONAL PARK



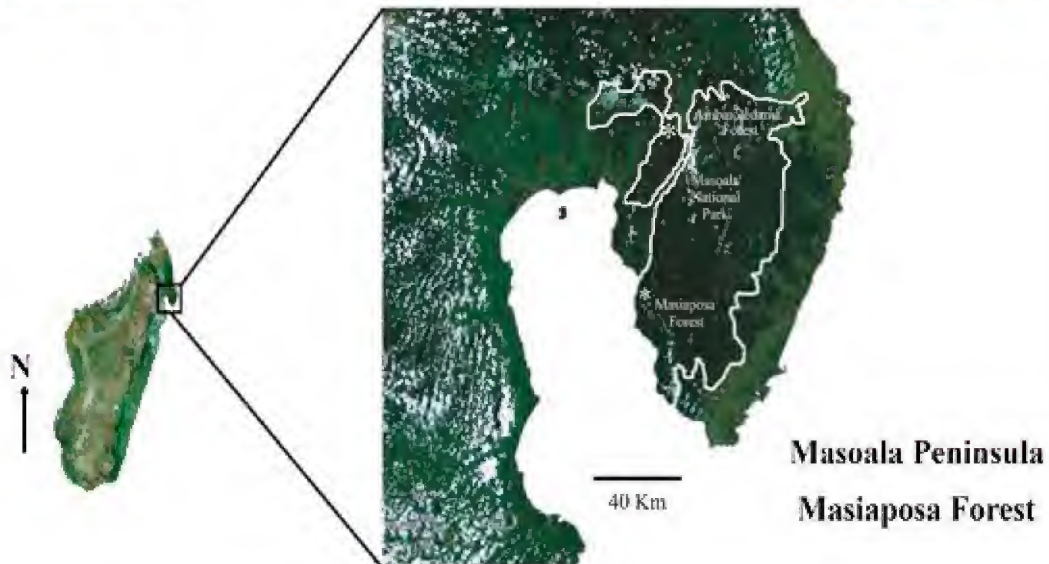
Moore's Woolly Lemur

Avahi mooreorum



Scott's Sportive Lemur

Lepilemur scottorum



Masoala Peninsula

Masiaposa Forest

RUNHUA LEI, SHANNON E. ENGBERG, RAMBININTSOA ANDRIANTOMPOHAVANA, SUSIE M. MCGUIRE,
RUSSELL A. MITTERMEIER, JOHN R. ZAONARIVELO, RICK A. BRENNEMAN, AND EDWARD E. LOUIS, JR.

Front cover: Distribution of two new species of nocturnal lemurs at Masoala National Park, Madagascar. Descriptions of Moore's woolly lemur, *Avahi mooreorum*, and Scott's sportive lemur, *Lepilemur scottorum*, are presented in this publication. Figure created by Shannon E. Engberg, Rick A. Brenneman, and Edward E. Louis, Jr.

SPECIAL PUBLICATIONS

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Nocturnal Lemur Diversity at Masoala National Park

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Nocturnal Lemur Diversity at Masoala National Park

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NOCTURNAL LEMUR DIVERSITY AT MASOALA NATIONAL PARK

RUNHUA LEI, SHANNON E. ENGBERG, RAMBININTSOA ANDRIANTOMPOHAVANA, SUSIE M. MCGUIRE, RUSSELL A. MITTERMEIER, JOHN R. ZAONARIVELO, RICK A. BRENNEMAN, AND EDWARD E. LOUIS, JR.

ABSTRACT

Approximately 3,000 base pairs of mitochondrial DNA sequence data were utilized as part of a phylogenetic analysis to investigate the taxonomic relationships of the nocturnal lemurs (genera *Avahi* and *Lepilemur*) of Masoala National Park. The molecular data distinguished all currently described *Avahi* and *Lepilemur* species, but it also revealed previously unrecognized biodiversity within these genera at Masoala National Park. Diagnoses of a new species of woolly lemur and a new species of sportive lemur from Masoala National Park are presented. These results demonstrate that cryptic species can be revealed through molecular data and the importance of initiating further detailed studies in previously unstudied sites to confirm the distributions of all species, particularly nocturnal lemur species.

Key words: *Avahi*, D-loop, *Lepilemur*, Madagascar, Masoala National Park, Pastorini fragment

INTRODUCTION

Madagascar, with a land mass of 587,000 km² spanning from latitudes 12°S to 25°S, has a diverse range of habitats created by an interaction of variable abiotic factors such as temperature and rainfall (north-south gradient) and altitude (gradients that segregate the island into generally eastern and western sections (Donque 1972; Stevens and O'Connor 2006)). Based on high levels of endemism and species diversity but confronted by persistent human related pressures, Madagascar is considered one of the most critical global priorities for conservation protection and has been designated as one of the world's most important biodiversity hotspots (Green and Sussman 1990; Myers 2000; Groombridge and Jenkins 2002). Prominently figured into conservation efforts, the lemurs of Madagascar are protected under the Convention of International Trade of Endangered Species (CITES) and are designated by the IUCN/SSC Red List Categories from critically endangered to threatened or as data deficient (IUCN 2004). Directed almost entirely towards nocturnal lemurs, recent molecular and morphological studies have led to a significant increase in the number of recognized

species (Groves 2000; Rasoloarison et al. 2000; Thalmann and Geissmann 2000, 2005; Kappeler et al. 2005; Andriaholinirina et al. 2006; Andriantompohavana et al. 2006, 2007; Louis et al. 2006a, 2006b; Olivieri et al. 2007). Given the numerous taxonomic revisions and newly described species that have been presented over the past decade, a comprehensive understanding of the distribution and taxonomy of the nocturnal lemurs is needed. We contribute to this line of research by presenting data on the taxonomy of two nocturnal genera, *Lepilemur* and *Avahi*, concentrating on the systematics of these taxa at Masoala National Park.

The Masoala Peninsula, located in northeastern Madagascar, contains one of the largest remaining tracts of humid evergreen forest, including significant areas of lowland forest (Fig. 1; Kremen et al. 1999). During an initial lemur survey of the peninsula, Sterling and Rakotoarison (1998) identified nine species including *Lepilemur mustelinus* and *Avahi laniger*. Conducting rapid lemur surveys of Masoala National Park, Vasey (2000) and Mittermeier et al. (2006) also identified nine

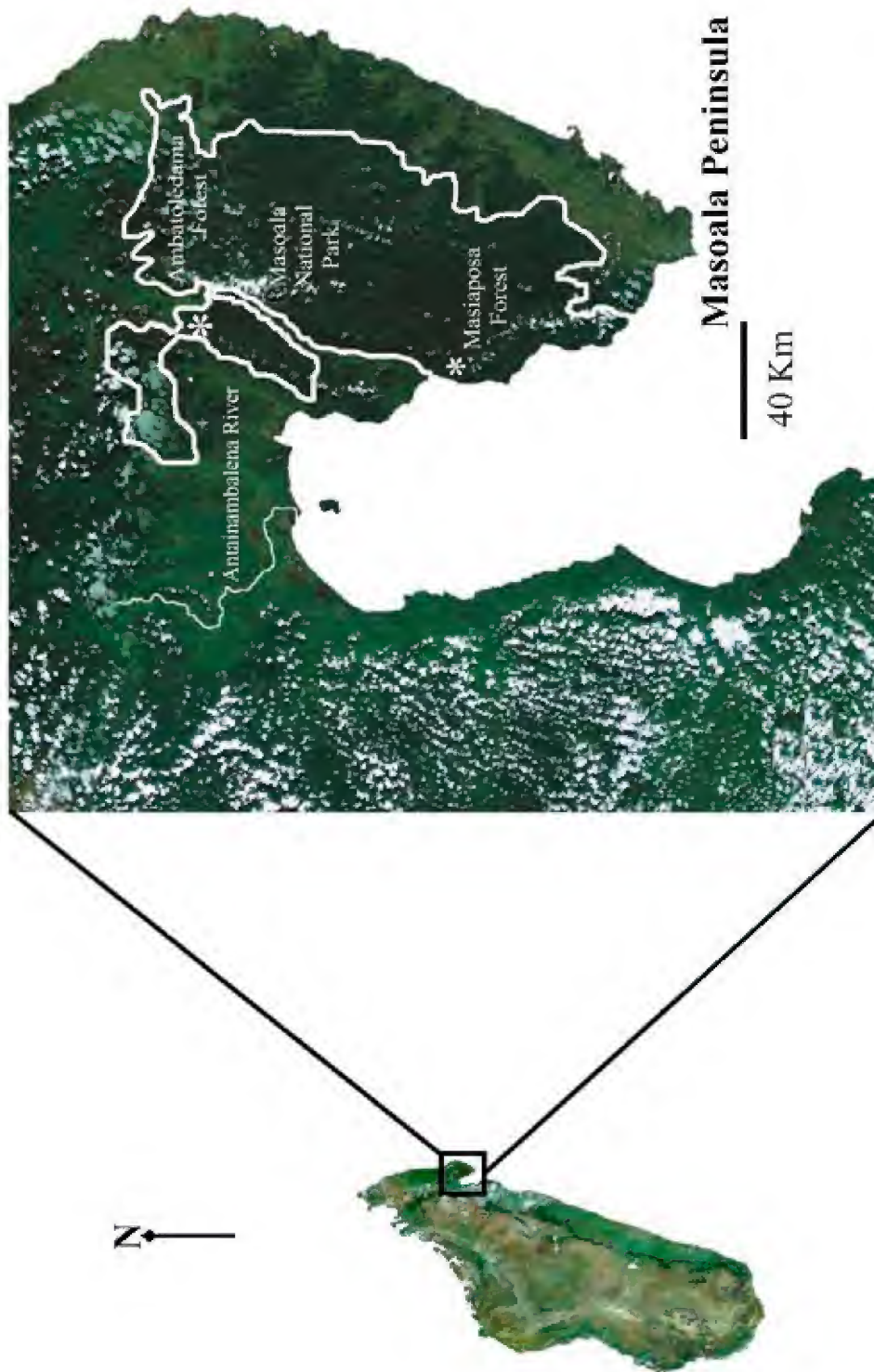


Figure 1. Map of Masoala National Park, Madagascar. Samples collected at Masiaposa Forest were analyzed in this publication along with data from Andriantompohavana et al. (2007) and Louis et al. (2006b; Appendix II(a)).

species of lemurs, including the same two nocturnal lemurs. Ganzhorn et al. (2006), Louis et al. (2006b), Craul et al. (2007), and Oliveri et al. (2007), illustrated the importance of Madagascar's rivers as barriers to migration, promoting the processes of allopatric speciation. The Antainambalana River, a prominent river to the west of Masoala Peninsula, is known to be a barrier to several species such as *Propithecus diadema* to *Propithecus candidus*, and *Varecia variegata* to *Varecia rubra* (Fig. 1). Furthermore, Simpson (1964) described the potential isolating effects of peninsulas on fauna and flora, citing as an example the Florida peninsula. On peninsulas, there is an inverse relationship between the number of species and geographic distance from the mainland, which acts as a reservoir of genetic diversity. This potential isolating effect has been documented with *Varecia rubra* in the Masoala Peninsula, but its significance has not been demonstrated with other taxa. Given the potential significance of rivers and peninsulas on speciation, the taxonomic status of the *Lepilemur* and *Avahi* species should be established. Therefore, a molecular analysis was conducted in order to clarify the nocturnal lemur diversity of the genera *Avahi* and *Lepilemur* at Masoala National Park.

The genus *Avahi* was originally described as a single species, *Avahi laniger*, with two subspecies: *A. laniger occidentalis* in northern, northwestern, and western forests, and *A. laniger laniger* in the eastern forests (Petter et al. 1977; Tattersall 1982). Subsequently, based on cytogenetic studies, Rumpler et al. (1990) elevated them to full species status as *A. occidentalis* and *A. laniger*. According to morphological and vocalization data, Thalmann and Geissmann (2000) described a third species, *A. unicolor*, from the Ampasindava Peninsula, and more recently discovered a fourth species, *A. cleesei*, from the Tsingy de Bemaraha region (Thalmann and Geissmann 2005). Further revisions of this genus by Zaramody et al. (2006) and Andriantompohavana et al. (2007) expanded the number of recognized species in the genus *Avahi* to eight full species. Based on the distribution of woolly lemurs, the *Avahi* species found in the Masoala Peninsula has been understood to be the eastern woolly lemur, *Avahi laniger*.

The sportive lemurs, genus *Lepilemur*, are medium-sized, nocturnal lemurs found in virtually all forested areas in Madagascar (Mittermeier et al. 2006).

Initially, Schwarz (1931) and Hill (1953) recognized two species of *Lepilemur*: *L. mustelinus* from the eastern rainforests and *L. ruficaudatus* from the western and southern dry forests of Madagascar. *L. ruficaudatus* was further divided into two subspecies, *L. ruficaudatus ruficaudatus* and *L. ruficaudatus leucopus*. Since then, the taxonomy of the sportive lemurs has been revised repeatedly (Petter and Petter-Rousseaux 1960; Rumpler and Albignac 1975; Petter et al. 1977; Tattersall 1982; Jenkins 1987; Mittermeier et al. 1994; Ravoarimanana et al. 1999, 2004; Thalmann 2000; Groves 2001; Rumpler et al. 2001; Thalmann and Ganzhorn 2003). More recently, the genus *Lepilemur* has undergone a dramatic expansion to 24 recognized species with the taxonomic revisions of Andriaholinirina et al. (2006; described three new species), Louis et al. (2006b; described 11 new species), Rabarivola et al. (2006; described one new species), and Craul et al. 2007; described two new species). Additionally, Zinner et al. (2007) raised important concerns with the sportive lemurs recently described in northwestern Madagascar, citing the need for comparative analyses including the holotype specimens (specifically *L. dorsalis* and *L. grandidieri*) and respective data sets, along with an indepth morphological analysis. Based on the distribution of sportive lemurs, the *Lepilemur* species found in the Masoala Peninsula has been understood to be the weasel sportive lemur, *Lepilemur mustelinus*.

Historically, sympatric reproductive isolation, described in the Biological Species Concept (BSC), predominantly was used as criterion defining species (Mayr 1942). However, when the putative species is a geographically isolated or an allopatrically defined population, this concept is difficult to implement. The Phylogenetic Species Concept (PSC) not only encompasses a cladistic perspective, but also allows the scientist to focus operationally on the results of evolution to present and to delineate species (Cracraft 1983; Wheeler and Platnick 2000; Louis et al. 2006a).

In this paper, we present comparative phylogenetic analyses of mitochondrial DNA sequence, augmented with morphological and phenotypic character data, of the nocturnal lemurs from the genera *Avahi* and *Lepilemur* at Masoala National Park to recently recognized woolly (Andriantompohavana et al. 2007) and sportive lemurs (Louis et al. 2006b). The results of phylogenetic analyses of molecular sequence data

for the genera *Lepilemur* and *Avahi* reveal unique biodiversity at Masoala National Park, Madagascar. Diagnostic descriptions of this previously undescribed lemur diversity are presented. Additionally, an electronic database that includes all *Avahi* and *Lepilemur* field data and photographs, including data for the paratype specimens, is curated at the Museum of Texas Tech Uni-

versity. The database is stored in the Type Specimen Collection in multiple media formats. This collection of field data and photographs, as well as additional tables and figures, also are available online at the website of Omaha's Henry Doorly Zoo. See Appendices I-III for a directory of appropriate website addresses.

METHODS

Sampling.—All lemurs investigated in this study were wild-caught and were immobilized with a CO₂ projection rifle or blowgun with 10 mg/kg of Telazol (Fort Dodge Animal Health; Overland Park, KS; Appendix I(a-b)). All recently recognized woolly and sportive lemurs were included in the analysis except for *L. mittermeieri* (Rabarivola et al. 2006). Rabarivola et al. (2006) utilized cytogenetic and cytochrome b subunit sequence data in their analyses, and since no sample was available to generate comparative sequence data, we could not include this species in this study. Four 2.0 mm biopsies and 1.0 cc per kilogram of whole blood were collected from each sedated animal and immediately stored in room temperature storage buffer (Longmire et al. 1992). We recorded the location of the immobilized lemurs using a global positioning system (Appendix I(a-b)). Beginning in 2000, while the animal was sedated, we placed a HomeAgain (Schering-Plough Veterinary Corp.; Kenilworth, NJ) microchip subcutaneously between the scapulae of each lemur (Appendix I(a-b)). This procedure was used to field catalog each animal with a unique recognition code to provide for the capability to re-identify positively all captured individuals during any future immobilizations.

Measurements were taken on the sedated animals as described in Andriantompohavana et al. (2007) and Louis et al. (2006b). For presentation purposes, we present the weight, head crown, body length, and tail length in this publication following the guidelines of Smith and Jungers (1997; Appendix I(a)). All other measurements, e-voucher photographs, and data are available at <http://www.omahazoo.com/ccr/index.asp?page=ccr/genetics/genhome.htm>. All traits were tested for significance among species using ANalysis Of VAriance (ANOVA) performed using an online statistical service (Kirkman 1996). Species means

were plotted in histogram form with the appropriate 95% confidence intervals.

As previously discussed in Thalmann and Geissmann (2005), Andriantompohavana et al. (2006), Louis et al. (2006a, 2006b), and Andriantompohavana et al. (2007), the utilization of whole vouchers as the designated holotype for a new species is not a prerequisite; however, opportunistic collections can later supplement morphological and/or molecular data in combination with curated blood and/or tissue samples. The woolly and sportive lemurs are prime candidates for this methodology since the highly folivorous dietary requirements of these groups of lemurs currently precludes any attempts to curate "live vouchers" (Thalmann and Geissmann 2005; Louis et al. 2006b; Andriantompohavana et al. 2007). Total genomic DNA for the three paratype specimens of *Avahi* and *Lepilemur* species are currently curated at the Museum of Texas Tech University (TK145356; TK145357; TK145358; and TK145333; TK145334; TK145335, respectively).

Data Collection.—Genomic DNA was extracted from a 2.0 millimeter ear punch using phenol-chloroform extraction (Sambrook et al. 1989; Appendix I(b)). To correlate our data with previously published molecular studies, we analyzed the following regions of the mitochondrial DNA (mtDNA): the displacement loop or control region (D-loop; Baker et al. 1993; Wyner et al. 1999); the 12S subunit ribosomal RNA gene, along with a portion of the tRNA^{Phe} (only the *Lepilemur* individuals; 12S rRNA; Hedges 1994; Wyner et al. 1999); and a fragment of the cytochrome oxidase subunit III gene (COIII), NADH-dehydrogenase subunits 3, 4L, and 4 (ND3, ND4L, and ND4), as well as the tRNA^{Gly}, tRNA^{Arg}, tRNA^{His}, tRNA^{Scr}, and partial tRNA^{Leu} genes (subsequently referred to as the PAST fragment; Pastorini et al. 2000). Using 50 nanograms of genomic

DNA, the D-loop and the PAST (2386 bp) fragments were amplified using the following conditions: 94°C for 30s, 47°C for 45s, 72°C for 45s for 34 cycles. Since potential nuclear insertions or mitochondrial pseudogenes within the nuclear genome can be amplified inadvertently, we chose to minimize this likelihood by amplifying both mitochondrial DNA regions as intersecting or overlapping segments (Zhang and Hewitt 1996). Consequently, the PAST fragment was generated from five amplified segments. Additionally, to eliminate further amplification of nuclear insertions, a rapid, efficient, and species independent technique derived from the degenerate oligonucleotide-primed PCR method (DOP-PCR) was used to generate the PCR products (Telenius et al. 1992). Adapting this LL-DOP-PCR (long products from low quantity), the sequence data were generated from overlapping segments to confirm the consensus D-loop and PAST fragments. The samples were electrophoresed in a 1.2% agarose gel to verify the PCR product and purified using QIAquick PCR purification kit (QIAGEN; Valencia, CA). The cleaned products were cycle sequenced using a big dye-terminator sequencing kit (Applied Biosystems; Foster City, CA). The sequences were analyzed by capillary electrophoresis with an Applied Biosystems Prizm 3100 genetic analyzer. A suite of internal sequencing primers from Pastorini et al. (2000) and Pastorini et al. (2001) were used to generate the PAST fragment. Additionally, PCR and sequencing primers specific for *Avahi* and *Lepilemur* were designed for the PAST and D-loop fragments (Louis et al. 2006b; Andriantompohavana et al. 2007). The sequence fragments were aligned to generate a consensus sequence using Sequencher (Gene Corp; Ann Arbor, MI), and the consensus sequences were aligned using Clustal X (Thompson et al. 1997). All sequences have been deposited in GenBank and the sequence data and information are available from the referenced accession numbers (Appendix I(a-b)). The sequence alignments for the data sets are available from the first author upon request.

Phylogenetic Analysis.—Maximum-parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) analyses were performed for the phylogenetic study of the D-loop, PAST, 12S rRNA (*Lepilemur* only), and combined fragments (D-loop and PAST fragments for *Avahi* and *Lepilemur*) sequence data with PAUP* 4.0b10 software (Swofford 2001). The trees described in this paper are all consensus trees except for the bootstrap analysis (all trees were presented as

phylograms for presentation purposes only). Bootstrap analyses were accomplished with 1000, 1000, 3000, and 4000 replicates with the D-loop, 12S rRNA, PAST, and D-loop/PAST sequence files, respectively, with 10 random addition heuristic searches per replicate option selected. Only nodes with greater than 50% support were reported. The D-loop NJ tree was generated using the Tamura-Nei model (Tamura and Nei 1993). The stepwise addition option was selected for MP and ML analyses, and corrections for nucleotide sequence data suggested by Kimura (1980) were used with the NJ analyses. Gaps were considered as a fifth character in MP analyses, whereas gaps were treated as missing data in the NJ analyses. The ML trees were estimated via the heuristic search. For the substitution model, the transition/transversion ratios were estimated in MacClade 3.01 (Maddison and Maddison 1992) and a discrete approximation to gamma distribution was estimated for among site rate variation. The default settings were maintained for all other settings, thus yielding the equivalent of the HKY model (Hasegawa et al. 1985). In addition to character-based phylogenetic analysis of DNA sequences, PAUP* 4.0b10 software (Swofford 2001) and MEGA 3.1 (Kumar et al. 2004) were used to calculate uncorrected pairwise distances ('p') and Kimura distance measures for D-loop and PAST fragments.

Bayesian inference analyses were conducted using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The model of evolution was selected by using Mrmodeltest 2.2, a modified version of Modeltest 3.6 (Posada and Crandall 1998; Nylander 2004). A Markov Chain Monte Carlo (MCMC) run with four simultaneous chains and 1,000,000 generations was performed. Every hundredth generation, the tree with the best likelihood score was saved, resulting in 4,000 trees. The 4,000 trees were condensed in a majority rule consensus tree using PAUP* 4.0b10 (Swofford 2001). Branch supports were assigned as posterior probabilities on the consensus tree. The pattern of sequence evolution was estimated by conducting a minimum spanning network generated with the program NETWORK 4.11 (Bandelt et al. 1999; Forster et al. 2001) and Arlequin 2.0 (Schneider et al. 2000).

As described in Davis and Nixon (1992), Wyner et al. (1999), Mayor et al. (2004), Andriantompohavana et al. (2006), Louis et al. (2006a, 2006b), and Andrian-

tompohavana et al. (2007), we utilized MacClade 3.01 (Maddison and Maddison 1992) and MEGA3.1 (Kumar et al. 2004) to designate evolutionary significant units (ESU) for the genera *Avahi* and *Lepilemur* using a population aggregate analysis (PAA) of the D-loop and PAST sequence data. With the sequential addition of each individual without an *a priori* species design-

ation, a PAA distinguishes attributes or apomorphic characters according to the smallest definable unit (Davis and Nixon 1992; Ravaoarimanana et al. 2004; Mayor et al. 2004; Andriantompohavana et al. 2006; Louis et al. 2006a, 2006b; Andriantompohavana et al. 2007; Olivieri et al. 2007).

RESULTS

Population Aggregate Analysis for the genera Avahi and Lepilemur.—A summary of the PAA for the diagnostic sites for the genera *Avahi* and *Lepilemur* are condensed in Tables 1A-B and Tables 1C-E, respectively. The results from the PAA of the D-loop and PAST sequence data for the genus *Avahi* are presented as diagnostic nucleotide sites in Appendices III(a-b), respectively. The results from the PAA of the D-loop, 12S rRNA, and PAST sequence data for the genus *Lepilemur* are presented as diagnostic nucleotide sites in Appendices III(c-e), respectively. Multiple diagnostic characters differentiated each *Avahi* species, along with two types of *A. laniger* (only the results from the PAST fragment PAA designated attributes for the two types), three types of *A. peyrierasi*, and previously unrecognized biodiversity, *Avahi* species *nova* #4 from Masoala National Park (Tables 1A-B). Furthermore, multiple diagnostic characters differentiated each recognized *Lepilemur*, along with distinguishing two distinct clades designated as *Lepilemur* species *nova* #1 at Masoala National Park and *Lepilemur* species *nova* #2 from Mananara-Nord National Park (Tables 1C-E).

Morphological Data of the genera Avahi and Lepilemur.—Means and standard deviations as well as P-values for significant differences among species are presented in Table 2A-C for the *Avahi* species and Table 3A-C for the *Lepilemur* species. The histograms, depicting the mean and 95% confidence interval of significant tests detecting morphometrical trends among the species within a genus, and those morphological units in which significant differences were identified are presented (*Avahi* species *nova* #4, *Lepilemur* species *nova* #1, and *Lepilemur* species *nova* #2 were analyzed as separate taxa; Appendix II(a-d)). The raw morphological and field data for woolly and sportive lemur species are presented in Appendix I(a).

Phylogenetic Analysis of the genus Avahi.—Mitochondrial DNA sequence data were completed for two fragments, D-loop and PAST (approximately 3,000 bp) for 119 individuals, representing all eight recognized species of woolly lemurs from a total of 14 sites (Appendices I(a-b); Andriantompohavana et al. 2007). Based on the phylogenetic reconstructions of the NJ, MP, and ML analyses of three sequence alignments (D-loop, PAST, and D-loop-PAST combined), the eight recognized *Avahi* species are differentiated and could be delineated into two major terminal clades or subgroups (Fig. 2; Appendices II(e-i)). The first subgroup corresponds to the woolly lemurs of western Madagascar as follows: *A. occidentalis*, *A. cleesei*, and *A. unicolor* (Fig. 2). The second subgroup corresponds to the eastern woolly lemurs as follows: *A. laniger*, *A. meridionalis*, *A. ramanantsoavana*, *A. peyrierasi*, and *A. betsileo* (Fig. 2). Three haplotype groups or types of *A. peyrierasi* were distinguishable within the woolly lemurs at Ranomafana National Park (Fig. 2; Appendices II(e-i)). In addition, another group of woolly lemurs from Masoala National Park, *A. species nova* #4, formed an apparent clade from the other species (Fig. 2; Appendices II(e-i)). High bootstrap values support the MP, NJ, and Bayesian analyses with respect to the topology of the genera and species (Fig. 2; Appendices II(f, h, and i)). The minimum spanning network presents diagrammatically the speciation among eight woolly lemurs, along with *A. species nova* #4 (Fig. 3).

The uncorrected 'p' distance and the absolute genetic differences for D-loop and PAST fragments for the genus *Avahi* are presented in Appendix III(f-g). The highest average uncorrected pairwise distances and absolute genetic differences for the D-loop sequence alignment were between the western and eastern woolly

(text continued on page 19)

Table 1A. Summary of Population Aggregate Analysis (PAA) D-Loop fragment diagnostic sites for Avahi. Refer to Appendix III, Table 1A. *No character or attribute is available for this fragment.

Species	Fragment Size (bp)	PAA base pair location
<i>Avahi peyrierasi</i> type #2	562	172, 194, 195, 333
<i>Avahi peyrierasi</i> type #3	562	336, 548
<i>Avahi peyrierasi</i> type #1	562	349
<i>Avahi betsileo</i>	562	20, 227, 277, 309, 521
<i>Avahi meridionalis</i>	562	16, 64, 159, 422, 506
<i>Avahi ramanantsoavana</i>	562	158, 194, 199, 525
<i>Avahi laniger</i> type #1 (Mantadia)	562	543
<i>Avahi laniger</i> type #2 (Mananara-Nord)	562	*
<i>Avahi species nova</i> #4	562	154, 331
<i>Avahi unicolor</i>	563	106, 288, 397, 401
<i>Avahi cleesei</i>	563	64, 275, 276, 287, 300, 302, 332
<i>Avahi occidentalis</i>	563	295, 296, 314, 499

Table 1B. Summary of Population Aggregate Analysis (PAA) PAST fragment diagnostic sites for Avahi. Refer to Appendix III, Table 1B. The locality of *Avahi laniger* type #1 is Mantadia National Park. The locality of *Avahi laniger* type #2 is Mananara-Nord National Park. The locality of *Avahi species nova* #4 is Masoala National Park. *No character or attribute is available for this fragment.

Species	Fragment Size (bp)	PAA base pair location
<i>Avahi peyrierasi</i> type #2	2364	14, 57, 155, 344, 572, 698, 945, 1124, 1139, 1341, 1353, 1525, 1537, 1818, 1835, 1886, 1960, 2059, 2111, 2122
<i>Avahi peyrierasi</i> type #3	2364	54, 246, 701, 1408, 1584, 1861, 1946, 2096, 2153
<i>Avahi peyrierasi</i> type #1	2364	620, 683, 1426, 1783, 2165
<i>Avahi betsileo</i>	2364	920, 1053, 1837
<i>Avahi meridionalis</i>	2364	85, 289, 291, 339, 708, 847, 935, 952, 958, 1060, 1114, 1131, 1270, 1336, 1339, 1459, 1612, 1771, 2013, 2356
<i>Avahi ramanantsoavana</i>	2364	117, 279, 938, 940, 972, 985, 1261, 1273, 1381, 1384, 1407, 1807
<i>Avahi laniger</i> type #1	2364	671, 957, 1117
<i>Avahi laniger</i> type #2	2364	576, 835
<i>Avahi species nova</i> #4	2364	288, 650, 844, 957, 997, 1009, 1114, 1141, 1157, 1183, 1288, 1362, 1597, 1675, 1729, 1846, 1933, 1963, 1996, 2011, 2029, 2101, 2141, 2170, 2187, 2210
<i>Avahi unicolor</i>	2364	6, 93, 114, 133, 501, 636, 916, 1052, 1135, 1279, 1454, 1849, 1872, 2032, 2116, 2131
<i>Avahi cleesei</i>	2364	8, 118, 213, 258, 522, 704, 928, 1057, 1213, 1273, 1552, 1585, 1786
<i>Avahi occidentalis</i>	2364	47, 111, 121, 500, 540, 665, 764, 783, 826, 1075, 1138, 1168, 1333, 1477, 1519, 1525, 2023, 2053, 2116, 2138, 2332

Table 1C. Summary of Population Aggregate Analysis (PAA) D-Loop diagnostic sites for Avahi. Refer to Appendix III, Table 1C. The locality of *Lepilemur species nova* #1 is Masoala National Park. The locality of *Lepilemur species nova* #2 is Mananara-Nord National Park. *No character or attribute is available for this fragment.

Species	Fragment Size (bp)	PAA base pair location
<i>Lepilemur ankaranensis</i>	540	*
<i>Lepilemur milanoii</i>	540	130
<i>Lepilemur tymenlatchsoni</i>	538	117
<i>Lepilemur septentrionalis</i>	536	33, 37, 98, 111, 249
<i>Lepilemur dorsalis</i>	540	536, 537
<i>Lepilemur sahamalazensis</i>	542	*
<i>Lepilemur petteri</i>	534	*
<i>Lepilemur leucopus</i>	535	19
<i>Lepilemur ruficandatus</i>	535	103, 126, 249, 310, 535
<i>Lepilemur hubbardorum</i>	535	242, 253, 270, 302
<i>Lepilemur raudrianasoli</i>	538	33, 272
<i>Lepilemur edwardsi</i>	545-546	127, 139, 538
<i>Lepilemur grewcockorum</i>	544	139, 195, 357
<i>Lepilemur ahmansonorum</i>	542	*
<i>Lepilemur ayeclis</i>	537-538	21
<i>Lepilemur mustelinus</i>	552-553	*
<i>Lepilemur jamesorum</i>	552	132
<i>Lepilemur betsileo</i>	553	272, 273, 286
<i>Lepilemur fleuretae</i>	550	10, 24, 37, 287, 288, 314, 317, 330
<i>Lepilemur microdon</i>	530	25, 34, 107, 110, 120, 121, 123, 124, 125, 137, 139, 396, 554
<i>Lepilemur wrightae</i>	551	55, 58, 275, 301, 476, 493
<i>Lepilemur seali</i>	550	54, 159, 221
<i>Lepilemur species nova</i> #1	550	87, 160, 195, 231, 327, 475
<i>Lepilemur species nova</i> #2	550	24, 30, 140, 187, 266

Table 1D. Summary of Population Aggregate Analysis (PAA) 12S rRNA diagnostic sites for *Lepilemur*. Refer to Appendix III, Table 1D. The locality of *Lepilemur species nova* #1 is Masoala National Park. The locality of *Lepilemur species nova* #2 is Mananara-Nord National Park. *No character or attribute is available for this fragment.

Species	Fragment Size (bp)	PAA base pair location
<i>Lepilemur ankaranensis</i>	855	*
<i>Lepilemur milanoii</i>	855	498
<i>Lepilemur tymerlachsoni</i>	855	394, 693, 854
<i>Lepilemur septentrionalis</i>	855	18, 60, 110, 154, 334, 488
<i>Lepilemur dorsalis</i>	855	761, 796
<i>Lepilemur sahamalazensis</i>	855	294, 766
<i>Lepilemur petteri</i>	855	240
<i>Lepilemur leucopus</i>	855	*
<i>Lepilemur ruficaudatus</i>	855	433
<i>Lepilemur hubbardwom</i>	855	340
<i>Lepilemur randrianasoli</i>	855	6, 242, 369, 391, 418, 666
<i>Lepilemur edwardsi</i>	854	*
<i>Lepilemur grewcockorum</i>	854	*
<i>Lepilemur ahmansonorum</i>	855	413, 641, 723, 768
<i>Lepilemur aeeclis</i>	855	389, 556
<i>Lepilemur mustelinus</i>	854	*
<i>Lepilemur jamesorum</i>	854	66
<i>Lepilemur betsileo</i>	853	432
<i>Lepilemur fleuretae</i>	854	53, 500, 757
<i>Lepilemur microdon</i>	855	235, 270, 757, 786
<i>Lepilemur wrightae</i>	854	349, 746
<i>Lepilemur seali</i>	855	186, 322, 544
<i>Lepilemur species nova</i> #1	855	245, 326
<i>Lepilemur species nova</i> #2	855	126, 241, 799

Table 1E. Summary of Population Aggregate Analysis (PAA) PAST fragment diagnostic sites for *Lepilemur*. Refer to Appendix III, Table 1E. The locality of *Lepilemur species nova* #1 is Masoala National Park. The locality of *Lepilemur species nova* #2 is Mananara-Nord National Park. *No character or attribute is available for this fragment.

Species	Fragment Size (bp)	PAA base pair location
<i>Lepilemur ankaranensis</i>	2359-2360	364, 858, 1315, 1804
<i>Lepilemur milanoii</i>	2359	342, 769, 1896
<i>Lepilemur tymerlachsoni</i>	2361	152, 1309, 1378, 1861, 1898, 1995
<i>Lepilemur septentrionalis</i>	2361	44, 113, 211, 214, 274, 353, 354, 533, 551, 555, 576, 674, 734, 1103, 1174, 1231, 1347, 1399, 1448, 1492, 1550, 1582, 1603, 1630, 1777, 2144, 2146, 2363
<i>Lepilemur dorsalis</i>	2360	579, 717, 746, 1525, 1780, 2163, 2168, 2177, 2236
<i>Lepilemur sahamalazensis</i>	2360	204, 399
<i>Lepilemur petteri</i>	2360-2361	337, 578, 779, 957, 1615
<i>Lepilemur leucopus</i>	2360	220, 448, 719, 836, 1960
<i>Lepilemur ruficaudatus</i>	2361	94, 127, 365, 776, 1074, 1370, 1783, 1835, 1867, 1921
<i>Lepilemur hubbardorum</i>	2360	350, 543, 566, 629, 681, 1012, 1015, 1240, 1396, 1559, 1906, 1907, 2111
<i>Lepilemur randrianasoli</i>	2360	191, 699, 849, 923, 982, 1018, 1035, 1053, 1432, 1444, 1753, 1981, 1988, 2250, 2267
<i>Lepilemur edwardsi</i>	2360	1018, 1114, 1343, 1474, 1979
<i>Lepilemur grewcockorum</i>	2360	406, 888, 896, 988, 1114, 1226, 1354, 1537, 2230
<i>Lepilemur ahmansorum</i>	2360	46, 304, 350, 1096, 1097, 1402, 1818, 2141, 2170
<i>Lepilemur aeeclis</i>	2360	535, 548, 563, 581, 975, 1357, 1368, 1423, 1442, 1990, 2089, 2107
<i>Lepilemur mustelinus</i>	2359-2360	85, 982
<i>Lepilemur jamesorum</i>	2360	2144
<i>Lepilemur betsileo</i>	2360	8, 1057
<i>Lepilemur fleuretae</i>	2360	29, 103, 269, 358, 534, 546, 553, 664, 1124, 1574, 2013, 2023
<i>Lepilemur microdon</i>	2361	146, 510, 581, 596, 826, 829, 1171, 1369, 1954, 1991, 2077, 2164
<i>Lepilemur wrightae</i>	2360	133, 663, 691, 871, 907, 942, 1058, 1117, 1120, 1837, 1856, 1936, 2041, 2096, 2181, 2185, 2331
<i>Lepilemur seali</i>	2361	124, 147, 290, 626, 665, 692, 722, 1302, 1313, 1371, 1679, 1875, 1879, 1969
<i>Lepilemur species nova</i> #1	2361	86, 567, 1157, 1337, 1435, 1483, 1606, 2165
<i>Lepilemur species nova</i> #2	2361	72, 256, 871, 1033, 1112, 1167, 1237, 1336, 1538, 1902

Table 2A. Morphometric data collected from sedated Avahi individuals. (Individual morphological data available online; see Appendix Ia).

Species Name	Common Name	N	Weight (kg)	Head Crown (cm)	Body Length (cm)	Tail Length (cm)
<i>Avahi cleesei</i>	Cleese's woolly lemur	5	0.86±0.11	7.3±1.2	24.4±4.0	32.2±2.4
<i>Avahi occidentalis</i>	Western woolly lemur	8	0.94±0.10	6.4±0.5	22.4±1.2	34.2±3.3
<i>Avahi unicolor</i>	Sambirano woolly lemur	6	0.84±0.12	6.5±0.2	22.0±1.6	33.3±2.1
<i>Avahi laniger</i>	Eastern woolly lemur	24	1.13±0.25	6.9±0.6	22.8±1.9	33.0±3.8
<i>Avahi peyrierasi</i> type #1	Peyrieras' woolly lemur (Type #1)	7	0.96±0.10	6.8±0.5	21.0±1.5	31.3±1.6
<i>Avahi peyrierasi</i> type #2	Peyrieras' woolly lemur (Type #2)	11	1.08±0.12	8.1±1.5	21.6±1.9	32.8±3.4
<i>Avahi peyrierasi</i> type #3	Peyrieras' woolly lemur (Type #3)	6	1.11±0.21	7.0±0.5	23.2±3.5	30.5±3.8
<i>Avahi ramanantsoavana</i>	Ramanantsoavana's woolly lemur	10	0.98±0.13	7.1±1.0	23.2±2.0	32.4±6.4
<i>Avahi meridionalis</i>	Southern woolly lemur	10	1.06±0.17	6.5±0.1	24.2±1.5	33.4±3.7
<i>Avahi betsileo</i>	Betsileo woolly lemur	9	1.05±0.16	7.6±1.6	22.1±2.3	31.4±2.9
<i>Avahi</i> species nova #4	woolly lemur new species #4	10	0.92±0.11	6.6±0.7	24.1±1.7	33.3±3.9

Table 2B. Morphometric data collected from sedated Avahi individuals. (Individual morphological data available online; see Appendix Ia).

Species Name	Forelimb				Hindlimb					
	Thumb Length (cm)	LD Length (cm)	Hand Length (cm)	Ulna/ Radius Length (cm)	Humerus Length (cm)	Hallux Length (cm)	LD Length (cm)	Foot Length (cm)	Tibia Length (cm)	Femur Length (cm)
<i>Avahi cleesei</i>	2.6±0.2	3.0±0.1	5.8±1.3	8.5±0.7	6.5±0.3	4.9±0.2	2.9±0.2	8.6±1.4	11.7±0.4	13.2±0.4
<i>Avahi occidentalis</i>	2.0±0.2	2.7±0.2	6.3±0.4	7.6±0.4	6.1±0.6	3.9±0.8	2.4±0.5	8.3±0.3	10.2±0.9	12.4±0.3
<i>Avahi unicolor</i>	2.3±0.2	3.0±0.4	6.4±0.3	8.3±0.4	5.9±0.6	3.9±0.5	3.1±0.1	8.7±0.3	10.3±0.3	12.6±0.6
<i>Avahi laniger</i>	2.6±0.4	3.5±0.5	7.2±0.7	8.6±0.8	6.5±0.8	5.1±0.4	3.2±0.5	9.8±0.6	11.4±1.0	13.2±1.3
<i>Avahi peyrierasi</i> type #1	2.0±0.1	3.1±0.1	6.9±0.1	8.2±0.4	5.6±0.2	3.3±0.2	3.0±0.1	9.4±0.3	10.3±0.2	13.8±0.3
<i>Avahi peyrierasi</i> type #2	2.2±0.5	3.2±0.3	6.1±1.6	8.6±0.6	6.7±1.0	4.0±1.1	3.1±0.2	8.8±1.4	11.6±1.3	14.2±0.8
<i>Avahi peyrierasi</i> type #3	1.9±0.6	3.4±0.5	7.4±0.7	8.1±0.8	5.6±1.1	3.7±0.4	3.0±0.0	9.9±0.6	10.8±0.9	13.9±1.0
<i>Avahi ramanantsoavana</i>	2.5±0.0	3.7±0.7	6.6±0.2	8.6±0.5	6.6±0.6	5.0±0.1	4.0±1.5	9.4±0.1	11.0±1.2	12.9±0.6
<i>Avahi meridionalis</i>	2.6±0.1	3.4±0.2	6.5±0.6	9.3±0.6	6.3±0.5	5.3±0.3	3.3±0.3	10.0±0.6	11.1±0.5	13.9±0.8
<i>Avahi betsileo</i>	2.6±0.3	3.3±0.2	5.5±1.9	8.6±0.7	6.2±0.8	5.1±0.3	3.2±0.3	8.6±1.6	11.4±1.4	13.4±0.9
<i>Avahi</i> species nova #4	2.5±0.5	3.4±0.9	6.8±0.7	8.7±0.6	7.0±0.5	4.7±0.9	3.5±0.9	9.9±0.6	11.8±0.8	12.2±1.3

Table 2C. Morphometric data collected from sedated Avahi individuals. Morphometric parameters, standard deviations and significance levels (* $P < 0.01$, ** $P < 0.001$) for 11 Avahi species and types. (Individual morphological data available online; see Appendix 1a).

Avahi Species	Weight **	Head *	Body length *	Tail length	Thumb **	Long digit **	Hand **	Radius/ Ulna **	Humerus *	Thumb **	Rear long digit *	Foot **	Tibia **	Femur **
<i>A. unicolor</i>	average 0.840	6.467	21.950	33.300	2.300	3.000	6.350	8.250	5.883	3.883	3.050	8.717	10.283	12.550
	stdev 0.134	0.175	1.750	2.333	0.245	0.405	0.321	0.409	0.611	0.492	0.152	0.279	0.371	0.653
<i>A. occidentalis</i>	average 0.944	6.350	22.350	34.188	2.025	2.713	6.263	7.575	6.100	3.888	2.438	8.263	10.238	12.363
	stdev 0.108	0.550	1.235	3.499	0.255	0.259	0.414	0.420	0.628	0.868	0.524	0.346	0.941	0.342
<i>A. cleesei</i>	average 0.860	6.720	22.400	32.240	2.620	3.020	6.380	8.520	6.500	4.940	2.900	8.620	11.700	13.220
	stdev 0.119	0.497	3.001	2.678	0.192	0.084	0.205	0.726	0.374	0.207	0.255	1.534	0.485	0.476
<i>A. mooreorum</i>	average 0.924	6.570	24.130	33.340	2.490	2.900	6.820	8.710	6.970	4.710	3.510	9.920	11.810	12.200
	stdev 0.110	0.660	1.676	3.902	0.517	0.387	0.750	0.597	0.452	0.917	0.945	0.553	0.758	1.345
<i>A. laniger</i>	average 1.166	6.917	23.026	33.483	2.565	3.596	7.304	8.670	6.604	5.113	3.283	9.852	11.548	13.309
	stdev 0.185	0.587	1.680	3.102	0.354	0.401	0.562	0.787	0.728	0.360	0.402	0.626	0.781	1.158
<i>A. betsileo</i>	average 1.075	6.400	22.120	31.360	2.630	3.330	7.033	8.610	6.150	5.080	3.170	8.640	11.360	13.420
	stdev 0.172	0.167	2.390	3.065	0.287	0.231	0.423	0.769	0.806	0.333	0.287	1.680	1.457	0.902
<i>A. peyrierasi</i> type #2	average 1.054	7.300	21.436	32.567	2.273	3.230	7.129	8.536	6.727	4.191	3.070	8.800	11.591	14.273
	stdev 0.133	0.400	1.938	3.357	0.573	0.291	0.243	0.609	1.058	1.264	0.263	1.425	1.306	0.764
<i>A. peyrierasi</i> type #1	average 0.957	6.829	21.014	31.329	2.000	3.100	6.871	8.229	5.557	3.343	2.957	9.386	10.286	13.771
	stdev 0.098	0.475	1.476	1.596	0.141	0.100	0.125	0.411	0.237	0.223	0.079	0.273	0.157	0.298
<i>A. peyrierasi</i> type #3	average 1.110	6.950	23.200	30.500	1.850	3.350	7.400	8.100	5.600	3.650	3.000	9.850	10.750	13.900
	stdev 0.213	0.495	3.504	3.835	0.636	0.495	0.707	0.849	1.131	0.354	0.000	0.636	0.919	0.990
<i>A. ramanantsoavana</i>	average 0.992	6.686	23.171	31.863	2.525	3.367	6.657	8.650	6.640	5.050	3.267	9.064	11.875	13.057
	stdev 0.124	0.951	1.860	4.349	0.050	0.306	0.382	0.442	0.564	0.191	0.551	0.719	1.284	0.493
<i>A. meridionalis</i>	average 1.060	6.450	24.170	33.410	2.620	3.410	6.460	9.300	6.340	5.260	3.310	10.040	11.130	13.860
	stdev 0.176	0.135	1.573	3.890	0.123	0.238	0.631	0.606	0.486	0.288	0.314	0.593	0.552	0.804

Table 3A. Morphometric data collected from sedated *Lepilemur* individuals (Individual morphological data available in Appendix Ia).

Species Name	Common Name	N	Weight (kg)	Head Crown (cm)	Body Length (cm)	Tail Length (cm)
<i>Lepilemur tymerlachsoni</i>	Hawk's sportive lemur	10	0.88±0.10	6.5±0.2	23.1±1.5	24.7±2.2
<i>Lepilemur aeeclis</i>	Antafia sportive lemur	9	0.87±0.17	7.5±1.1	23.9±2.0	25.5±0.9
<i>Lepilemur ahmansonorum</i>	Ahmanson's sportive lemur	4	0.61±0.15	6.2±1.2	20.6±1.6	23.8±0.7
<i>Lepilemur petteri</i>	Petter's sportive lemur	5	0.63±0.05	5.1±0.4	23.2±0.9	23.7±1.4
<i>Lepilemur randrianasoli</i>	Randrianasolo's sportive lemur	3	0.92±0.08	8.2±0.6	24.1±1.7	27.4±1.8
<i>Lepilemur ankaranensis</i>	Ankarana sportive lemur	30	0.78±0.12	7.0±0.8	21.8±1.9	27.7±2.1
<i>Lepilemur septentrionalis</i>	Northern sportive lemur	2	0.58±0.18	8.3±1.1	18.7±1.7	24.8±4.5
<i>Lepilemur milanoii</i>	Daraina or Swimming sportive lemur	11	0.71±0.07	5.8±0.4	20.0±0.9	24.3±1.6
<i>Lepilemur betsileo</i>	Betsileo sportive lemur	2	1.10±0.15	7.5±0.4	25.3±0.1	27.2±1.2
<i>Lepilemur dorsalis</i>	Grey-Backed sportive lemur	6	0.75±0.07	7.5±0.4	23.2±1.5	27.5±0.8
<i>Lepilemur edwardsi</i>	Milne-Edwards' sportive lemur	11	1.10±0.11	6.7±0.6	26.3±2.7	28.4±2.1
<i>Lepilemur fleuretae</i>	Fleurete's sportive lemur	1	0.80±0.00	7.1±0.0	25.8±0.0	29.0±0.0
<i>Lepilemur sahamalazensis</i>	Sahamalaza sportive lemur	2	0.70±0.07	6.3±0.1	19.6±0.3	23.7±2.7
<i>Lepilemur grewcockorum</i>	Grewcock's sportive lemur	2	0.90±0.00	6.2±0.0	26.0±1.0	29.5±0.8
<i>Lepilemur hubbardorum</i>	Hubbard's sportive lemur	7	0.99±0.18	7.6±1.4	24.1±0.8	24.0±1.0
<i>Lepilemur ruficaudatus</i>	Red-tailed sportive lemur	3	0.86±0.06	6.9±1.0	24.3±1.7	24.2±3.6
<i>Lepilemur jamesorum</i>	James' sportive lemur	8	1.05±0.08	8.0±0.3	27.1±2.7	30.0±1.7
<i>Lepilemur leucopus</i>	White-Footed sportive lemur	17	0.55±0.07	6.5±0.6	19.6±1.4	24.2±1.2
<i>Lepilemur mustelinus</i>	Weasel sportive lemur	27	0.99±0.21	7.9±1.4	25.8±4.0	25.2±2.1
<i>Lepilemur wrightae</i>	Wright's sportive lemur	5	1.15±0.12	8.1±1.5	26.5±1.6	25.7±1.8
<i>Lepilemur microdon</i>	Small-Toothed sportive lemur	12	1.07±0.32	10.6±2.4	22.6±1.0	24.2±2.6
<i>Lepilemur seali</i>	Seal's sportive lemur	5	0.95±0.09	7.5±0.9	27.5±1.4	26.0±1.4
<i>Lepilemur</i> species nova #1	sportive lemur new species #1	5	0.88±0.14	6.7±0.6	26.6±0.9	27.8±2.0
<i>Lepilemur</i> species nova #2	sportive lemur new species #2	1	0.95±0.00	6.8±0.0	26.4±0.0	27.5±0.0

Table 3B. Morphometric data collected from sedated *Lepilemur* individuals. (Individual morphological data available online; see Appendix Ia).

Species Name	Forelimb				Hindlimb					
	Thumb Length (cm)	LD Length (cm)	Hand Length (cm)	Ulna/Radius Length (cm)	Humerus Length (cm)	Hallux Length (cm)	LD Length (cm)	Foot Length (cm)	Tibia Length (cm)	Femur Length (cm)
<i>Lepilemur tymerlachsoni</i>	2.4±0.1	2.6±0.2	5.7±0.3	7.5±0.3	5.2±0.4	3.4±0.2	2.5±0.1	8.2±0.3	8.3±0.5	9.8±0.5
<i>Lepilemur aecilis</i>	2.4±0.3	2.7±0.6	5.7±0.5	7.7±0.5	5.8±0.5	3.5±0.5	3.0±1.2	8.8±0.5	8.9±1.3	10.0±1.0
<i>Lepilemur ahmansonorum</i>	2.6±0.2	2.7±0.3	5.4±0.3	7.1±0.5	5.7±0.7	4.6±0.3	2.5±0.1	7.9±0.4	8.2±0.5	9.4±0.7
<i>Lepilemur petteri</i>	1.9±0.1	2.3±0.2	4.9±0.4	6.3±0.6	5.1±0.5	3.2±0.3	2.2±0.1	7.7±0.4	8.8±0.8	7.8±0.3
<i>Lepilemur randrianasoli</i>	2.6±0.1	2.7±0.2	6.0±0.4	7.9±1.1	5.8±0.2	4.0±0.5	2.5±0.3	9.0±0.6	9.1±0.4	9.9±0.2
<i>Lepilemur ankaranensis</i>	2.5±0.3	3.0±0.9	5.1±0.7	6.8±0.5	5.4±0.7	4.2±0.7	3.0±1.4	7.7±0.7	8.3±0.9	9.5±0.6
<i>Lepilemur septentrionalis</i>	2.4±0.5	2.7±0.1	5.1±0.1	6.3±1.1	6.1±0.1	4.8±0.1	2.6±0.4	7.5±0.7	9.0±1.6	9.8±1.8
<i>Lepilemur niltanotii</i>	2.1±0.4	2.4±0.5	5.0±0.7	6.0±0.6	4.5±0.4	3.3±0.4	2.4±0.5	7.1±0.9	7.1±0.6	8.6±1.3
<i>Lepilemur betsileo</i>	3.1±0.2	3.0±0.0	6.8±0.4	8.8±0.4	7.1±0.7	5.2±0.5	3.1±0.3	9.8±0.4	10.0±0.7	11.7±0.4
<i>Lepilemur dorsalis</i>	2.6±0.3	3.0±0.2	5.7±0.2	7.8±0.1	5.7±0.3	3.6±0.3	2.8±0.1	8.6±0.4	9.1±0.3	10.1±0.4
<i>Lepilemur edwardsi</i>	2.0±0.6	2.5±0.4	5.6±0.4	6.9±0.8	5.7±0.5	2.9±0.5	2.3±0.3	7.9±0.3	8.9±1.0	9.8±1.1
<i>Lepilemur fleuretae</i>	2.7±0.0	2.7±0.0	5.5±0.0	7.8±0.0	5.4±0.0	4.9±0.0	3.2±0.0	9.5±0.0	8.9±0.0	11.3±0.0
<i>Lepilemur sahamalazensis</i>	1.6±0.1	2.2±0.2	4.8±0.4	6.4±0.1	4.3±0.3	2.6±0.1	1.9±0.0	7.1±0.1	7.5±0.1	9.3±0.4
<i>Lepilemur greweckorum</i>	2.3±0.1	2.4±0.0	5.1±0.1	6.7±0.1	5.7±0.1	3.1±0.1	2.3±0.4	7.7±0.1	7.6±0.5	10.1±0.0
<i>Lepilemur hubbardorum</i>	2.6±0.1	2.9±0.1	5.5±0.2	6.8±0.4	5.6±0.4	4.6±0.2	2.4±0.2	8.3±0.3	9.1±0.7	9.8±0.6
<i>Lepilemur ruficaudatus</i>	2.6±0.0	2.4±0.2	5.2±0.1	6.8±0.3	5.7±0.4	3.7±1.2	3.0±1.5	6.6±1.2	9.5±0.4	9.3±0.5
<i>Lepilemur jamesorum</i>	3.0±0.1	3.3±0.1	6.3±0.5	7.5±1.4	7.3±1.0	4.8±0.3	2.9±0.1	8.7±0.6	11.6±0.6	11.8±0.6
<i>Lepilemur leucopus</i>	1.9±0.3	2.3±0.3	4.8±0.3	6.0±0.6	4.5±0.5	3.0±0.5	2.2±0.3	7.2±0.4	7.8±0.8	8.9±0.4
<i>Lepilemur mustelinus</i>	2.6±0.3	3.6±1.4	6.2±0.4	8.0±0.7	6.4±0.7	4.8±0.7	2.9±0.4	9.1±0.4	9.7±0.9	10.9±0.9
<i>Lepilemur wrightae</i>	2.9±0.5	3.2±0.3	6.1±0.1	8.3±0.7	6.5±0.3	4.4±0.2	3.7±1.6	9.7±0.3	10.2±1.5	11.5±0.6
<i>Lepilemur microdon</i>	2.5±0.4	3.0±0.4	3.8±1.5	7.3±0.6	6.8±0.9	4.4±0.6	2.8±0.4	7.0±0.9	11.2±1.5	11.2±1.8
<i>Lepilemur seali</i>	2.2±0.3	3.2±0.4	6.2±0.2	8.4±0.3	6.2±0.3	3.9±1.2	3.2±0.6	9.2±0.3	10.4±0.8	10.6±0.8
<i>Lepilemur</i> species nova #1	2.1±0.3	2.9±0.2	5.9±0.3	8.4±0.4	6.3±0.3	3.5±0.2	2.8±0.3	8.3±1.3	9.3±2.0	8.7±0.5
<i>Lepilemur</i> species nova #2	2.6±0.0	3.1±0.0	6.4±0.0	9.1±0.0	6.1±0.0	4.0±0.0	3.9±0.0	9.3±0.0	8.6±0.0	10.7±0.0

Table 3C. Morphometric data collected from sedated *Lepilemur* individuals. Morphometric parameter averages and standard deviations among 19 *Lepilemur* species significant at $P < 0.001$. (Individual morphological data available online; see Appendix Ia).

Lepilemur Species	P < 0.0001	Weight	Head	Body Length	Tail Length	Thumb	Long Digit	Hand	Radius/Ulna	Humerus	Hind Thumb	Rear Long Digit	Foot	Tibia	Femur
<i>L. petersi</i>	average	627.0	5.080	23.240	23.720	1.940	2.260	4.880	6.300	5.100	3.240	2.240	7.680	8.840	7.820
	stdev	46.6	0.390	0.871	1.408	0.134	0.152	0.415	0.561	0.534	0.329	0.055	0.363	0.750	0.259
<i>L. hubbardorum</i>	average	994.3	7.150	24.057	24.029	2.643	2.857	5.471	6.800	5.629	4.586	2.443	8.343	9.057	9.843
	stdev	177.2	0.889	0.772	0.993	0.098	0.079	0.206	0.412	0.415	0.195	0.223	0.305	0.670	0.577
<i>L. randrianasoli</i>	average	916.7	8.200	24.100	27.400	2.600	2.733	6.000	7.900	5.833	4.033	2.500	9.033	9.100	9.867
	stdev	76.4	0.600	1.732	1.808	0.100	0.153	0.436	1.058	0.208	0.473	0.265	0.643	0.361	0.208
<i>L. ahmansonorum</i>	average	612.5	6.225	20.625	23.800	2.550	2.650	5.425	7.125	5.675	4.550	2.500	7.850	8.200	9.350
	stdev	149.3	1.195	1.567	0.739	0.191	0.342	0.330	0.499	0.750	0.265	0.141	0.436	0.462	0.714
<i>L. aeeclis</i>	average	822.7	7.538	21.944	25.222	2.444	2.422	5.278	7.333	5.644	3.400	2.556	8.189	8.467	9.578
	stdev	179.4	0.686	1.775	1.956	0.305	0.377	0.186	0.141	0.825	0.698	0.251	0.333	0.669	0.913
<i>L. tymerlachsoni</i>	average	875.0	6.480	23.140	24.650	2.400	2.620	5.670	7.530	5.160	3.430	2.520	8.210	8.250	9.810
	stdev	100.7	0.244	1.458	2.211	0.105	0.235	0.291	0.283	0.412	0.164	0.148	0.345	0.515	0.489
<i>L. edwardsi</i>	average	1100.0	6.682	26.273	28.445	2.036	2.527	5.645	6.936	5.718	2.927	2.255	7.900	8.850	9.755
	stdev	109.5	0.603	2.682	2.074	0.573	0.420	0.370	0.753	0.547	0.522	0.250	0.329	1.025	1.111
<i>L. grewockorum</i>	average	783.3	6.167	24.833	28.467	2.300	2.367	5.033	6.333	5.333	3.067	2.200	7.533	7.300	9.867
	stdev	202.1	0.058	2.139	1.790	0.100	0.058	0.153	0.551	0.643	0.115	0.265	0.306	0.557	0.404
<i>L. dorsalis</i>	average	733.2	7.300	22.767	27.500	2.467	2.900	5.650	7.850	5.633	3.667	2.767	8.550	9.083	10.033
	stdev	67.1	0.443	1.515	0.846	0.266	0.179	0.207	0.084	0.288	0.273	0.137	0.351	0.349	0.367
<i>L. ankaranensis</i>	average	778.1	6.913	21.677	27.697	2.477	2.971	5.221	6.783	5.353	4.171	2.550	7.706	8.352	9.506
	stdev	111.9	0.706	1.876	2.004	0.297	0.859	0.473	0.529	0.674	0.661	0.403	0.685	0.899	0.606
<i>L. milanoi</i>	average	713.9	6.333	21.467	26.013	2.273	2.587	5.353	6.627	5.120	3.593	2.587	7.953	7.807	9.260
	stdev	99.8	0.482	1.676	1.378	0.341	0.464	0.655	0.677	1.379	0.453	0.424	0.442	0.819	1.167
<i>L. seali</i>	average	961.7	7.683	25.700	26.540	2.200	3.250	6.283	8.250	6.117	3.900	3.267	9.133	10.517	10.600
	stdev	81.3	0.933	2.637	0.522	0.283	0.399	0.214	0.493	0.354	1.068	0.565	0.250	0.752	0.687
<i>L. scottorum</i>	average	876.0	6.720	26.600	27.840	2.080	2.900	5.860	8.380	6.280	3.540	2.800	8.340	10.120	8.740
	stdev	136.5	0.581	0.851	2.016	0.268	0.187	0.313	0.356	0.249	0.230	0.274	1.272	0.432	0.508
<i>L. ruficaudatus</i>	average	855.0	6.933	24.333	24.200	2.583	2.367	5.183	6.750	5.700	3.700	2.367	6.600	9.510	9.283
	stdev	57.7	1.002	1.686	3.617	0.029	0.208	0.144	0.250	0.361	1.217	0.289	1.249	0.373	0.539

Table 3C (cont.)

Lepilemur Species	P<0.0001	Weight	Head	Body Length	Tail Length	Thumb	Long Digit	Hand	Radius/ Ulna	Humerus	Hind Thumb	Rear Long Digit	Foot	Tibia	Femur
<i>L. mustelinus</i>	average	987.9	7.555	25.587	25.374	2.619	3.544	6.192	8.010	6.355	4.671	2.892	9.152	9.658	10.865
	stdev	201.2	1.002	3.794	2.066	0.304	1.295	0.364	0.685	0.644	0.705	0.364	0.421	0.893	0.866
<i>L. microdon</i>	average	1070.5	6.963	23.900	23.683	2.550	3.100	6.159	7.287	6.850	4.283	3.200	7.989	10.784	11.144
	stdev	320.4	0.895	2.180	2.543	0.568	0.374	1.193	0.427	0.889	0.611	0.654	1.416	1.361	1.288
<i>L. jamesorum</i>	average	1050.0	8.000	27.100	30.300	2.950	3.250	6.250	7.475	7.300	4.800	2.850	8.675	11.600	11.825
	stdev	77.5	0.283	2.722	1.664	0.071	0.071	0.465	1.403	1.015	0.283	0.071	0.568	0.594	0.556
<i>L. wrightae</i>	average	1150.0	7.375	25.240	25.460	2.700	3.080	6.140	8.060	6.300	4.240	2.825	9.600	10.080	11.540
	stdev	122.5	0.096	3.081	1.640	0.406	0.370	0.114	0.764	0.515	0.456	0.096	0.308	1.299	0.532
<i>L. leucopus</i>	average	528.9	6.437	19.500	23.995	1.879	2.289	4.768	5.895	4.374	2.932	2.158	7.205	7.647	8.742
	stdev	93.3	0.633	1.372	1.331	0.270	0.320	0.269	0.586	0.562	0.528	0.332	0.370	0.806	0.560

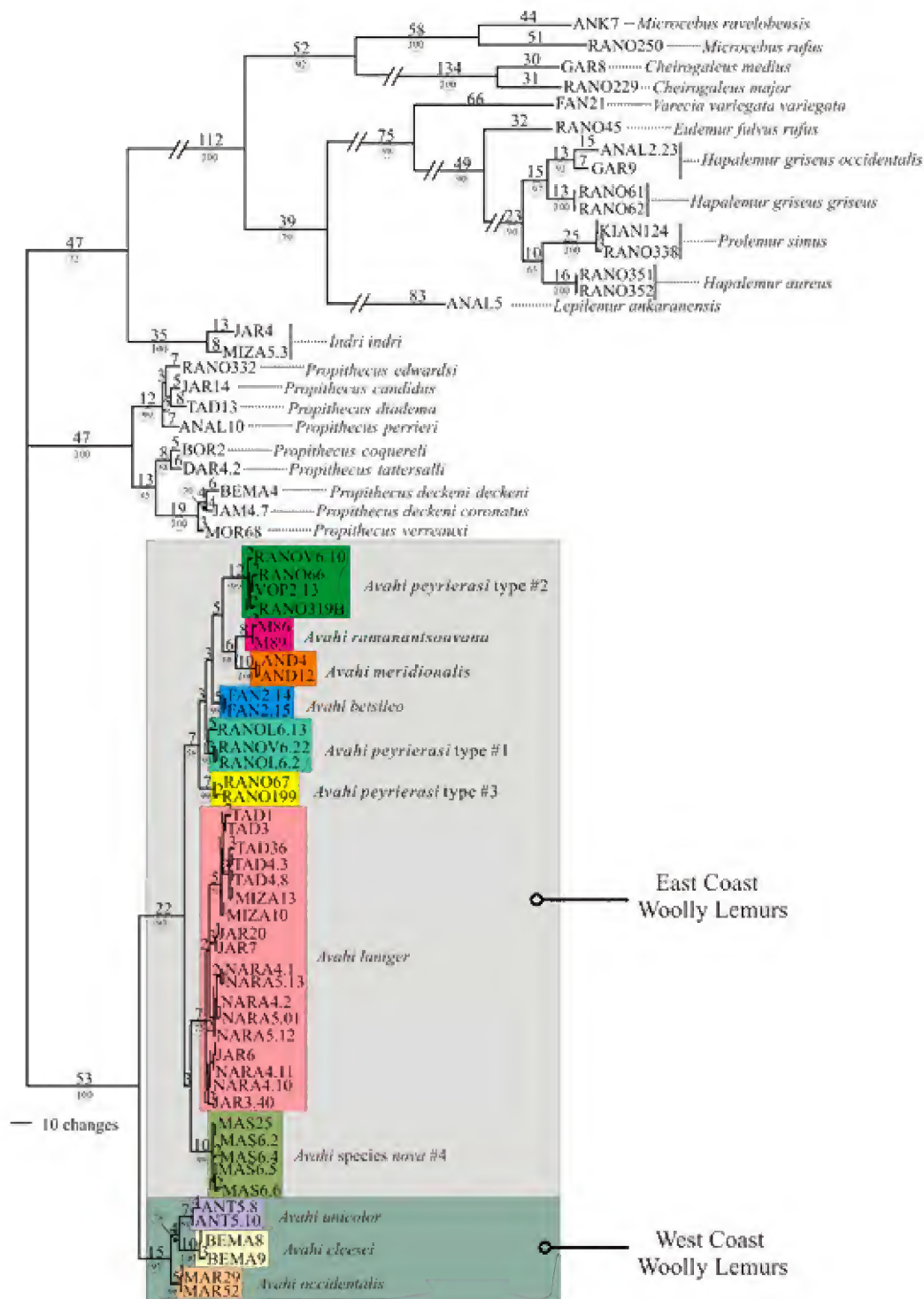
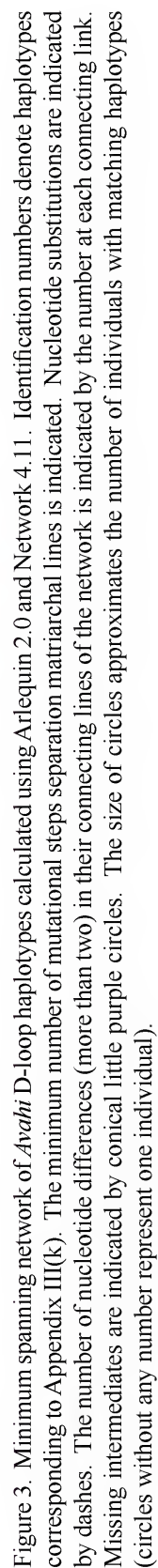


Figure 2. Maximum parsimony phylogram derived from the D-loop sequence data from 44 haplotypes from the 119 *Avahi* individuals with 26 out-group taxa (one of 4031 most parsimonious trees). Values above branches indicate number of changes between nodes. Values below branches indicate support of bootstrap pseudoreplicates. Length = 1540; CI = 0.5584; RI = 0.8618; RC = 0.4813; HI = 0.4416.



lemurs, ranging from 12.2 to 8.6 and 66 to 50 (*A. cleesei* to *A. peyrierasi* type #2, respectively). The highest average uncorrected pairwise distances and absolute genetic differences for the PAST sequence alignment were between the western and eastern woolly lemurs, ranging from 13.2 to 10.2 and 283 to 220, respectively.

Phylogenetic Analysis of genus Lepilemur.—Mitochondrial DNA sequence data were completed for three fragments, D-loop, 12S rRNA and PAST (approximately 3,800 bp) for 216 individuals, representing 22 recognized species of sportive lemurs from a total of 42 sites (Appendices I(a-b); Louis et al. 2006b). Based on the phylogenetic inferences of the NJ, MP and ML analyses of four sequence alignments (D-loop, 12S rRNA, PAST, and D-loop-PAST combined), the 22 recognized *Lepilemur* species are differentiated and divided into five terminal clades (Fig. 4; Appendix II(j-r)). The first terminal clade or subgroup consists of sportive lemurs from northern Madagascar as follows: *L. ahmansonorum*, *L. ankaranensis*, *L. milanoii*, *L. septentrionalis*, *L. tymerlachsoni*, *L. dorsalis*, and *L. sahamalazensis* (Fig. 4). The second terminal clade of sportive lemurs is associated with southern Madagascar and allied to the west-central subgroup (third terminal clade) as follows: *L. petteri* and *L. leucopus* (Fig. 4). The third terminal clade of sportive lemurs corresponds to west-central Madagascar as follows: *L. aeeclis*, *L. randrianasoli*, *L. ruficaudatus*, and *L. hubbardorum* (Fig. 4). The fourth terminal clade of sportive lemurs corresponds to eastern Madagascar as follows: *L. mustelinus*, *L. jamesorum*, *L. betsileo*, *L. fleuretae*, *L. wrightae*, *L. seali*, *L. species nova* #1, and *L. species nova* #2 (Fig. 4). The fifth subgroup included two western sportive lemurs and one eastern sportive lemur, *L. edwardsi* and *L. grewcockorum*, and *L. microdon*, respectively (Fig. 4). There is high bootstrap support for the MP, NJ, and Bayesian analyses with respect to the topology of the genera and species (Fig. 4; Appendix II(j, l, n, o, and r)).

The uncorrected 'p' distance and the absolute genetic differences for the genus *Lepilemur* are presented for D-loop, 12S rRNA, and PAST fragments in Appendix III(h-j), respectively. The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the D-loop sequence alignments between the sportive lemurs range from 18.6 to 2.7 and 107 to 20 (*L. species nova* #2 to *L. leucopus* and *L. ankaranensis* to *L. milanoii*, respectively). The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the 12S rRNA sequence alignment between the sportive lemurs range from 9.6 to 0.7 and 75 to 9, respectively (*L. randrianasoli* to *L. jamesorum* and *L. mustelinus* to *L. betsileo*, respectively). The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the PAST sequence alignment between sportive lemurs range from 20.8 to 1.2 and 415 to 33, respectively (*L. petteri* to *L. fleuretae* and *L. ankaranensis* to *L. milanoii*, respectively).

The phylogenetic inferences of the NJ and MP analyses for the combined data sets for the genera *Avahi* and *Lepilemur* are available as Appendices II(s-u). There is high bootstrap support for the MP and NJ analyses with respect to the topology of the genera and species. Appendices III(k-m) list the individual *Avahi* and *Lepilemur* haplotypes utilized in the various phylogenetic inferences. By including GenBank accessioned D-loop sequences and ND3 and ND4L sequences (Figs. 5 and 6, respectively, and Appendix III(m)) from Craul et al. (2007), *L. otto* was distinguishable from the other 22 sportive species, but *L. manasamody* was indistinguishable from *L. grewcockorum*. Additionally, the *Lepilemur* species *nova* #1 and *Lepilemur* species *nova* #2, along with the sportive lemur from Mahilaka (designated *L. dorsalis*), were distinct from the other sportive lemurs. The bootstrap support was high for the interspecific relationships; however, the intraspecific bootstrap was low. The minimum spanning network presents diagrammatically the speciation among 24 sportive lemurs (Fig. 7).

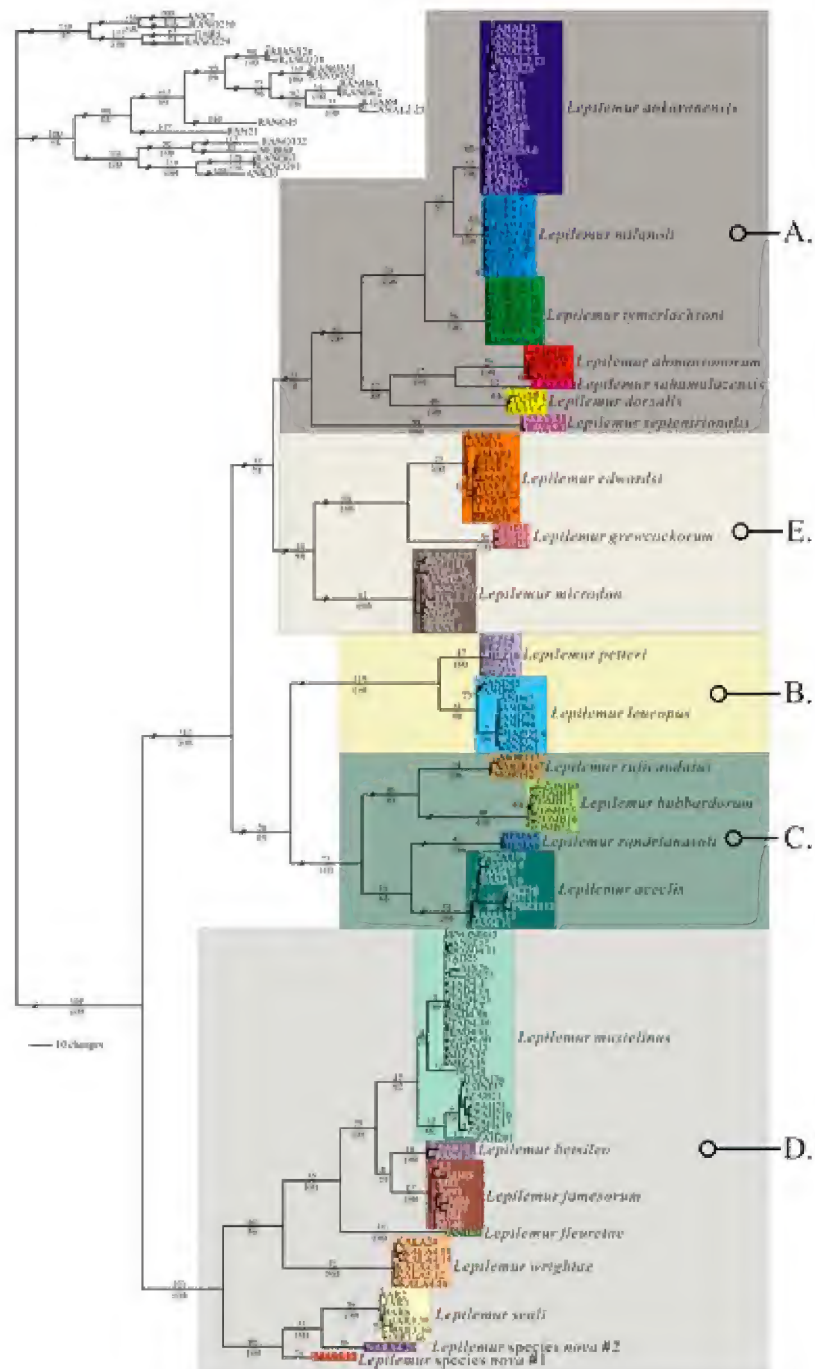


Figure 4. Maximum parsimony phylogram derived from the PAST sequence data from 158 haplotypes from the 216 *Lepilemur* individuals with 19 out-group taxa (one of 5158 parsimonious trees). Values above branches indicate number of changes between nodes. Values within circles along branches indicate support of bootstrap pseudoreplicates. Length = 5158; CI = 0.3806; RI = 0.9154; RC = 0.3484; HI = 0.6194. The subgroups of *Lepilemur* species is as follows: A. northern sportive lemurs; B. southern sportive lemurs; C. western sportive lemurs (west-central); D. eastern sportive lemurs; and E. western and eastern sportive lemurs.

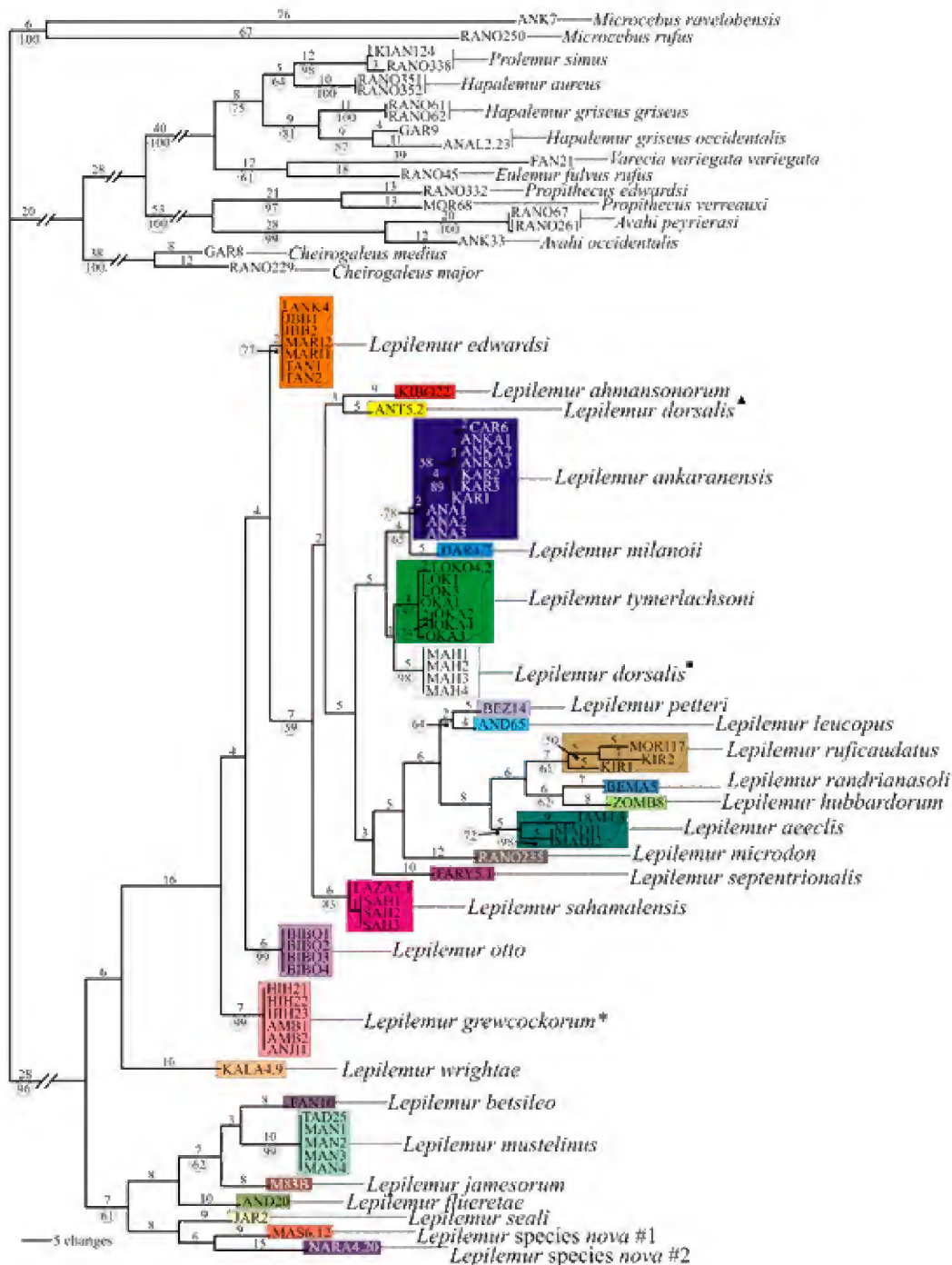


Figure 5. Maximum parsimony phylogram derived from the D-loop sequence data from 37 haplotypes from the 69 *Lepilemur* individuals with 19 out-group taxa (one of six most parsimonious trees). Values above branches indicate number of changes between nodes. Values within circles along branches indicate support of bootstrap pseudoreplicates. Length = 969; CI = 0.4334; RI = 0.8057; RC = 0.3492; HI = 0.5666. Accessed sequences utilized from Craul et al. (2007). *Designates GenBank accession sequences for *L. manasamody*. ▲Designates the *Lepilemur dorsalis* type at Antafondro and Manongarivo. ■Designates the *Lepilemur dorsalis* type at Mahilaka. GenBank accessions and base size information is available in Appendix III(m).

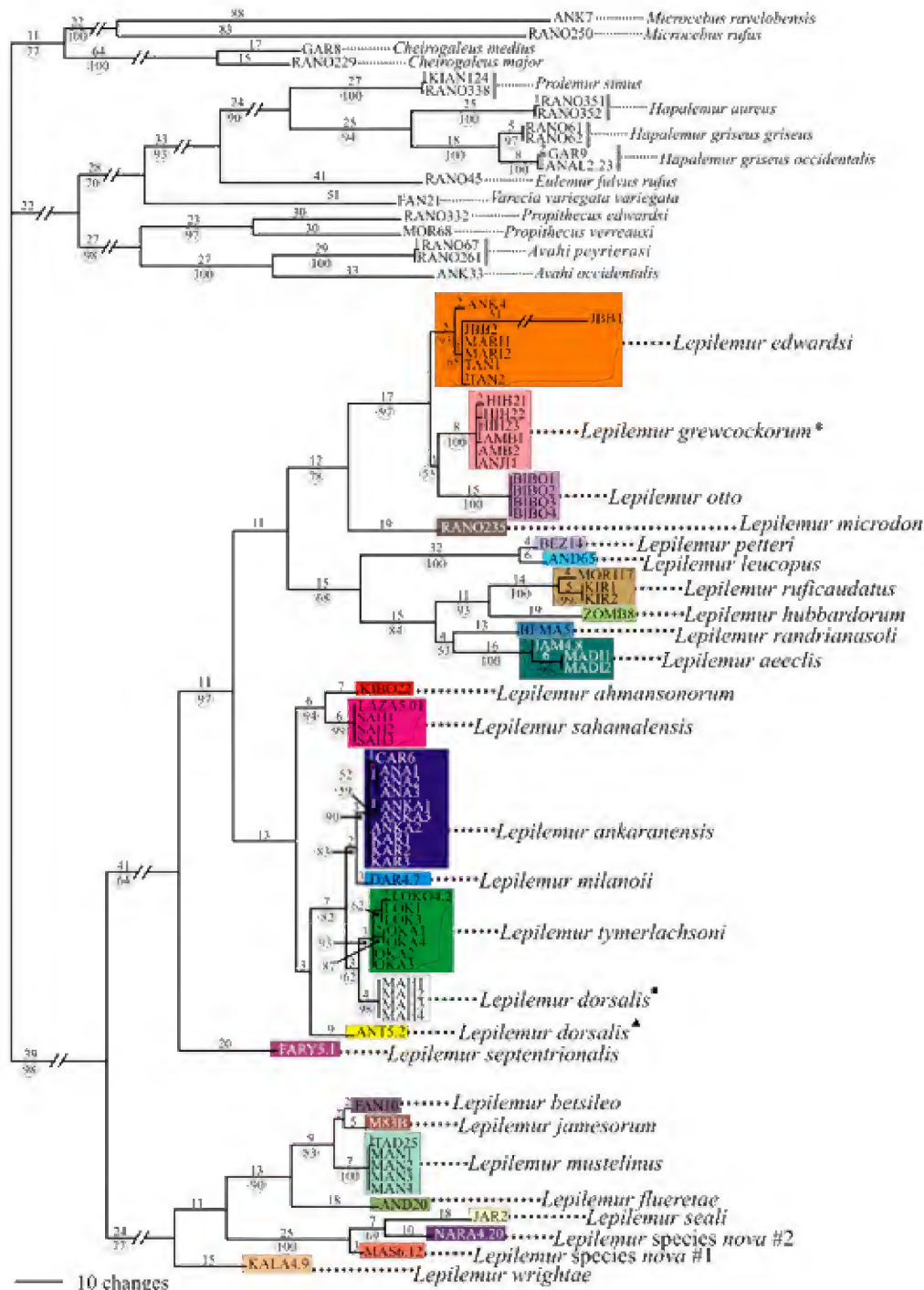


Figure 6. Maximum parsimony phylogram derived from the ND3 and ND4L sequence data from 41 haplotypes from the 69 *Lepilemur* individuals with 19 out-group taxa (one of 14 most parsimonious trees). Values above branches indicate number of changes between nodes. Values within circles along branches indicate support of bootstrap pseudoreplicates. Length = 1388; CI = 0.4135; RI = 0.8339; RC = 0.3449; HI = 0.5865. *Designates GenBank accession sequences for *L. manasamody*. ▲Designates the *Lepilemur dorsalis* type at Antafondro and Manongarivo. ■Designates the *Lepilemur dorsalis* type at Mahilaka. Accessioned sequences utilized from Craul et al. (2007). GenBank accessions and base size information is available in Appendix III(m).

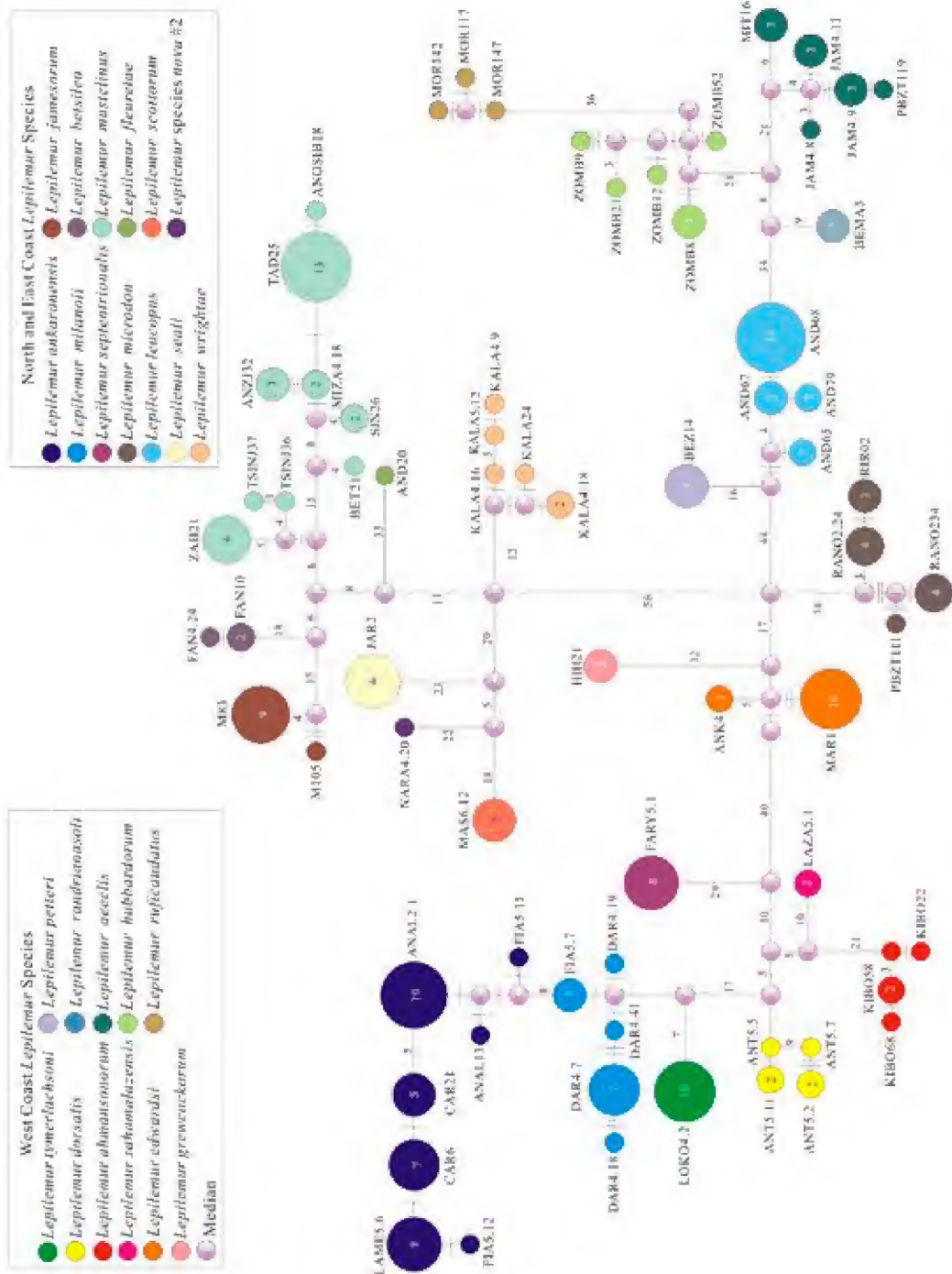


Figure 7. Minimum spanning network of *Lepilemur* D-loop haplotypes calculated using Arlequin 2.0 and Network 4.11. Identification numbers denote haplotypes corresponding to Appendix III(1). The minimum number of mutational steps separation matrarchal lines is indicated. Nucleotide substitutions are indicated by dashes. The number of nucleotide differences (more than two) in their connecting lines of the network is indicated by the number at each connecting link. Missing intermediates are indicated by conical little purple circles. The size of circles approximates the number of individuals with matching haplotypes (circles without any number represent one individual).

DISCUSSION

General Observations and Considerations.—All measurements were taken on live sedated animals, which potentially produce inherent error from multiple sources. Accurate age assessment by tooth size is often a problem in cases where the individual is a subadult, having not quite reached mature size, but no longer an obvious juvenile. Since expeditions are carried out in various habitats and forest fragments regardless of weather and/or convenience, seasonal differences can have an effect on the weights and measurements based on food availability and reproductive status (e.g., pregnant or lactating). In food abundant seasons, some anatomic reference points can be obscured by body condition. Furthermore, the accuracy of the morphological data requires the same researcher take all measurements over the length of a study, which was logistically impossible. Additionally, a larger and equivalent sample set is required to perform comprehensive and thorough morphological analyses. Working with live and subsequently released lemurs also precludes a morphological comparison to museum type vouchers. For these reasons, we interject the caveat to consider the measurements as rough baselines that demonstrate trends among the species of a genus providing the most accurate morphometric data that could be collected under field conditions.

Despite the limitations of the available morphological data, some observations can be made from such data. As Milne-Edwards (1875) suggested following early explorations of Madagascar, substantiated by Albrecht et al. (1990) and Godfrey et al. (1990), and which Zaonarivelo et al. (2007) found in limited numbers of *Avahi* species, we find the species inhabiting the western forests are on average smaller than the species inhabiting the east coast forests (Appendix II(a-b); Tables 2A-C). However, this generalization is not evident among the *Lepilemur* species (Appendix II(c-d); Tables 3A-C). We find that *L. leucopus* and *L. petteri* (both found on the southern tip of Madagascar) are on average the smaller of the sportive lemur species described to date. In addition, we determined that *L. jamesorum*, found in the Manombo Special Reserve, is more often larger in measurements than other species.

Although the type locality of *A. laniger* is considered to be Betanimena, Antongil Bay and Ma-

roanetsetra, this has not been conclusively determined (Jenkins 1987; Groves 2001; Zaramody et al. 2006; Andriantompohavana et al. 2007). As in Andriantompohavana et al. (2007), the phenotypic and molecular data (PAST fragment only) reveal two types of *A. laniger*, one type from Mantadia and another type from Mananara-Nord National Park and Anjanaharibe-Sud Special Reserve (Fig. 2; Appendix II(f, g, and i)). At this point, the authors consider the taxonomy of *A. laniger* unresolved at the species level. A comprehensive sample set incorporating the entire range from the Mangoro River to Anjanaharibe-Sud Special Reserve, along with representative data from the *A. laniger* type specimen, needs to be evaluated in order to determine species status and to assess whether the Onibe River and/or Manigory River act as the limit or barrier for both types or species.

Andriantompohavana et al. (2007) detected two types of *A. peyrierasi*. In this study, our data present three types of *A. peyrierasi* (Fig. 2; Appendix II(e-i)). All three types were sampled from forest fragments south and north of Ranomafana National Park and all types of *A. peyrierasi* can be found within the same forest fragment. One interpretation of the current sequence data is *A. peyrierasi* type #1, *A. peyrierasi* type #3, and *A. betsileo* represent one species complex, with *A. peyrierasi* #2 representing a distinct species as *A. peyrierasi*. Again, results generated from larger data sets over multiple generations, including pedigree and karyotype data, will be required to resolve the phylogenetic relationships between these three types of *A. peyrierasi*.

The phylogenetic inference of the three mtDNA regions, D-loop, 12S rRNA, and PAST fragments differentiated 23 species of sportive lemur, along with detecting unrecognized biodiversity in the sportive lemurs from Masoala and Mananara-Nord National Parks, *Lepilemur* species nova #1 and *Lepilemur* species nova #2 (Figs. 4-7; Appendices II(j-r)). The data corroborate the species status of *L. otto* in Craul et al. (2007), but it did not distinguish *L. manasamody* from *L. grewcockorum* (Figs. 5-6). The type localities of both species are only two kilometers apart with no discernible barrier (river or mountain; Zinner et al. 2007). Even though a comparison of all sportive lemurs except

for *L. mittermeieri* was conducted in this study, only partial GenBank sequences were available from other investigations (for D-loop, Craul et al. (2007) accessed 386-388 bp while Louis et al. (2006b) accessed 534-553 bp; for PAST, Craul et al. (2007) accessed 580-631 bp while Louis et al. (2006b) accessed 2359-2361 bp). Therefore, in both fragments, sequence data had to be truncated to enable comparative analyses, and this is reflected in poor bootstrap support for interspecific as opposed to intraspecific comparisons (Figs. 5-6; Appendix III(m)). Since *L. grewcockorum* (Louis et al. 2006b) was published prior to Craul et al. (2007), *L. grewcockorum* has priority over *L. manasamody*, the junior synonym. The sportive lemur from Mananara-Nord National Park, *Lepilemur* species *nova* #2, was initially grouped within the species *L. seali* in Louis et al. (2006b) due to the limited sample size. The addition of the Masoala sportive lemurs to the phylogenetic reconstruction and population aggregate analyses distinguished this individual from the population of sportive lemurs from Anjanaharibe-Sud Special Reserve (*L. seali*) and Masoala National Park (*Lepilemur* species *nova* #1).

In Zinner et al. (2007), the authors reviewed the current state of sportive lemurs of northwestern Madagascar, highlighting the confusion over the type locality of *L. dorsalis* and its generally accepted synonym, *L. grandidieri*. As shown in Figs. 5-6, multiple distinct populations of sportive lemurs are designated as *L. dorsalis* (Antafondro and Manongarivo refer to *L. dorsalis* (2) in Zinner et al. (2007), and Mahilaka individuals were not referenced in Zinner et al. (2007)). Although the authors of this study agree with the conclusions of Zinner et al. (2007) in that the holotypes of both *L. dorsalis* and *L. grandidieri* need to be utilized as comparative samples in molecular genetic analyses, several points should be clarified. First, the sportive lemurs from Antafondro and Manongarivo include identical haplotypes even though they are found on opposite sides of the Sambirano River, rather than from the south side of the river as stated by Zinner et al. (2007). Secondly, Zinner et al. (2007) did not employ from all available investigations comparative samples in their analyses, so not all of their conclusions or interpretations can truly reflect the phylogenetic relationships of the northwestern sportive lemurs (only D-loop sequence data was evaluated and only a subset from Louis et al. (2006b)).

Corroborating Evidence for Two New Lemur Species.—Multiple systematic studies utilizing molecular genetic analyses, specifically mtDNA sequence and/or cytogenetic data, have established this methodology to be a reliable approach in distinguishing and characterizing unique diversity, and in this case, novel lemur species (Rasoloarison et al. 2000; Andriaholinirina et al. 2006; Andriantompohavana et al. 2006; Louis et al. 2006a, 2006b; Rabarivola et al. 2006; Andriantompohavana et al. 2007; Olivieri et al. 2007). In this study, we examined the taxonomic status of *Avahi* and *Lepilemur* from Masoala National Park based on the Phylogenetic Species Concept (Wheeler and Platnick 2000; Mayor et al. 2004; Louis et al. 2006a, 2006b). Since the authors consider the morphometrical data as an insufficient sample set for the argument of establishing species status, it has been provided as only supplemental material. Although the morphological data showed that multiple characters differed significantly between both geographically distant and adjacent species (Appendices II(a-d); Tables 2C-3C), additional samples would be necessary to apply a comprehensive morphological analyses.

Three sets of criteria support the argument that the two new putative species of lemurs (one *Avahi* and one *Lepilemur*) should be considered distinct species. First, the absolute genetic distances and average uncorrected 'p' distances between geographically adjacent species and the proposed new species were within the limits of other conspecifics (Appendix III(f-j); Olivieri et al. 2007). Secondly, the population aggregate analysis (PAA) detected multiple diagnostic attributes for each terminal clade (Tables 1A-1E; Appendix III(a-e)). Invariably, as more individuals, populations, and species are included in the data set, the number of diagnostic attributes for each species will decrease due to random mutations at previously unique base positions in even more geographically distant species (Louis et al. 2006b; Andriantompohavana et al. 2007; Olivieri et al. 2007). Thirdly, these newly proposed species are located in the relative isolation of the Masoala Peninsula where other locally endemic taxa are to be found (e.g., *Varecia rubra*). Undoubtedly, additional morphometric data from museum vouchers, nuclear DNA sequence and genotype data, comprehensive samples from Mananara-Nord National Park, Anjanaharibe-Sud Special Reserve, and surrounding forest fragments, along with

behavioral, ecological, and acoustical data should be acquired to better characterize these new species.

Descriptive Diagnosis of Two New Species.—The phylogenetic inference of the mtDNA sequence data, along with the supplemental morphometric and phenotypic character data, differentiate the described species of woolly (genus *Avahi*) and sportive (genus *Lepilemur*) lemurs along with the previously unrecognized bioersivity from Masoala National Park for both genera, *Avahi* species *nova* #4 (NB #1, #2, and #3 are discussed in Andriantompohavana et al. (2007)) and *Lepilemur* species *novae* #1 and #2. Although the sportive lemur from Mananara-Nord, *Lepilemur* species *nova* #2, is considered distinct from other recognized sportive lemurs, until additional samples are available to confirm the reliability of the current information, the Mananara-Nord sportive lemur will not be designated as a proposed new species. A description of each new species is provided below.

***Avahi mooreorum*, New Species**
(formerly referred to as *Avahi* species *nova* #4)

Type Series.—Whole blood for MAS6.1 (TK145356; TTU-M 104519), adult female; MAS6.2 (TK145357; TTU-M 104520), adult male; and MAS6.3 (TK145358; TTU-M 104521), adult male; are stored and curated at Museum of Texas Tech University. Individual measurements, e-voucher photos, and collection data are given in *Avahi* Field Data Appendix I(a). MAS6.1, MAS6.2, and MAS6.3 were collected by Edward Louis, Richard Rakotonomenjanahary, Jean C. Randriamanana, Justin Andrianasolo, Philibert Randriamaronjaka, and Jean Claude Rakotoniaina on 20 November 2006 and 21 November 2006.

Type Locality.—Madagascar: Province de Antsiranana, Masoala National Park (approximately S15°40'008", E049°57'476").

Description.—*Avahi mooreorum* (0.92 kg) is slightly smaller than *A. laniger* (1.13 kg). The overall pelage is a mixture (mottled) of chocolate brown and light brown dorsum (Fig. 8). The ears are not readily seen, blending in with the rest of the head. It has a whitish patch under the mandible, and a distinct whitish patch, characteristic of other woolly lemurs, on the

posterior surface of the rear leg. *A. mooreorum* has a diffuse pattern without the noticeable eyebrow of *A. laniger*. The facemask is apparent, but not as pronounced as other *A. laniger* (east coast) types (Andriantompohavana et al. 2007). The dorsum is darker in coloration on the head as opposed to the distal portion of the dorsum (gradual lighter pattern towards the tail base, which is distinctly cream colored). The venter is gray including undersides of limbs. The tail is reddish-brown with a transition over the base to a cream color.

Diagnosis.—In the D-loop and PAST sequence fragments, *A. mooreorum* differs from the closest relative, *A. laniger*, by genetic and geographic distance $3.9\% \pm 0.8\%$ (28 informative sites) and $4.2\% \pm 0.4\%$ (104 informative sites), respectively. *A. mooreorum* has 28 diagnostic attributes (two attributes for D-loop and 26 attributes for PAST fragment).

Distribution.—Currently *A. mooreorum* is known only from Masoala National Park. Even though *A. laniger* has been confirmed in Anjanaharibe-Sud Special Reserve and Mananara-Nord National Park, further surveys are required to confirm the northern extent of *A. mooreorum* within Masoala National Park and possibly intervening forest fragments between Anjanaharibe-Sud Special Reserve and the Masoala Peninsula.

Comparisons and Remarks.—*A. mooreorum* (0.92 kg) is smaller in weight than *A. laniger* (1.13 kg), however, *A. mooreorum* is slightly larger in body length than *A. laniger*.

Etymology.—The name *mooreorum* is proposed in honor of the Moore Family - Gordon and Betty Moore, Ken and Kris Moore and Steve and Kathleen Moore - in recognition of their long-term commitment to biodiversity and conservation. The major support that they have provided through the Gordon and Betty Moore Family Foundation and other channels has been critical to advancing conservation in some of the world's most important and most endangered biodiversity hotspots, including Madagascar, and continues to have enormous impact.

Vernacular Names.—Moore's woolly lemur or Masoala woolly lemur.



Figure 8. *Avahi mooreorum*, Moore's woolly lemur. Photo by Edward E. Louis, Jr.

Lepilemur scottorum, New Species

(formerly referred to as *Lepilemur* species nova #1)

Type Series.—Whole blood for MAS6.12 (TK145333; TTU-M 104516), adult female; MAS6.13 (TK145334; TTU-M 104517), adult female; and MAS6.14 (TK145335; TTU-M 104518), adult male; are stored and curated at the Museum of Texas Tech University. Individual measurements, e-voucher photos, and collection data are given in *Lepilemur* Field Data Appendix I(a). MAS6.12, MAS6.13 and MAS6.14 were collected by Edward Louis, Richard Rakotonomenjanahary, Jean C. Randriamanana, Justin Andrianasolo, Philibert Randriamaranjaka, and Jean Claude Rakotoniaina on 20 November 2006.

Type Locality.—Madagascar: Province de Antsiranana, Masoala National Park (approximately S15°40'246", E049°57'537").

Description.—*Lepilemur scottorum* is a medium-sized sportive lemur (0.88 kg) with a uniform reddish-brown color pattern (Fig. 9). The venter is reddish brown. The pelage is long and thick throughout the body, with a diffuse black stripe, midline of the dorsum that ends mid-body. The face is whitish-gray. The cheeks and eyebrows are white. The hands and feet are reddish brown. The tail is reddish brown at the base and progressively brownish-gray towards the tip. The tip is black in coloration.

Diagnosis.—In the D-loop, 12S rRNA, and PAST sequence fragments, *L. scottorum* differs from its closest relatives, *L. seali* and *L. species nova* #2, by genetic and geographic distance $6.3\% \pm 1.1\%$ (36 informative sites) and $7.0\% \pm 1.2\%$ (37 informative sites), $2.2\% \pm 0.5\%$ (18 informative sites) and $1.8\% \pm 0.5\%$ (15 informative sites), and $5.1\% \pm 0.5\%$ (116 informative sites) and $4.2\% \pm 0.4\%$ (95 informative sites), respectively. *L. scottorum* has 16 diagnostic attributes (six attributes for D-loop, 2 attributes for 12S rRNA, and 8 attributes for PAST fragment).

Distribution.—Currently known only from Masoala National Park, Masiaposa Forest.

Comparisons and Remarks.—*Lepilemur scottorum* (0.88 kg) is smaller than *L. seali* (0.96 kg), *L. mustelinus* (0.99 kg), and *L. wrightae* (1.15 kg). Additional survey work is required to determine the eastern and northern range of *L. scottorum*.

Etymology.—The name *scottorum* is proposed in honor of the Suzanne and Walter Scott Jr. Family Foundation. Suzanne and Walter Scott Jr. are definitive leaders, mentors, and motivators of *in situ* and *ex situ* conservation throughout the world and have volunteered extensively in Henry Doorly Zoo's conservation programs for decades. Walter Scott, Jr. is a 32-year member of the Board of Directors of the Omaha Zoological Society. Formerly president of the board, he has been Chairman of the board since 1984. He is a philanthropist, business leader, and community activist on the board of many charitable organizations and businesses. Suzanne M. Scott, a long-time zoo supporter, in 1984 became the founding executive director of the Omaha Zoo Foundation responsible for establishing the zoo's endowment funds and raising money for special projects.

Vernacular Names.—Scott's sportive lemur.

In conclusion, Madagascar has lost approximately 90% of its original forest due to human activities such as agriculture and logging. Exacerbating this loss, the remaining forests have become increasingly isolated, resulting in an unconnected mosaic of forest fragments (Ganzhorn et al. 2001). The extreme levels of deforestation have left many species susceptible to stochastic events or anthropogenic pressures (Jernvall and Wright 1998; Ezard and Travis 2006). Moreover, there is insufficient information available on distributions of described lemurs across Madagascar. The current distributions of the genera *Avahi* (nine species) and *Lepilemur* (24 species) are presented in Figs. 10 and 11, respectively. The impact of rivers as barriers and the remarkable influence of isolation on allopatric speciation is diagrammatically demonstrated in Fig. 12. The inter-river system (IRS) for the genera *Microcebus* and *Lepilemur* in northwestern Madagascar as described by Olivieri et al. (2007) and Craul et al. (2007), respectively, can be vividly expanded across the island as unique paired biodiversity exists for the genera *Avahi* and *Lepilemur* wherever they are found. It is urgent to identify all biodiversity and species' distributions before the 2008 commitment to triple the amount of protected area by the Malagasy government. This data can only enhance the delineation of the reserve matrix by providing valuable information for wildlife and conservation agencies and allowing precise management decisions to be implemented to protect the existing biodiversity.

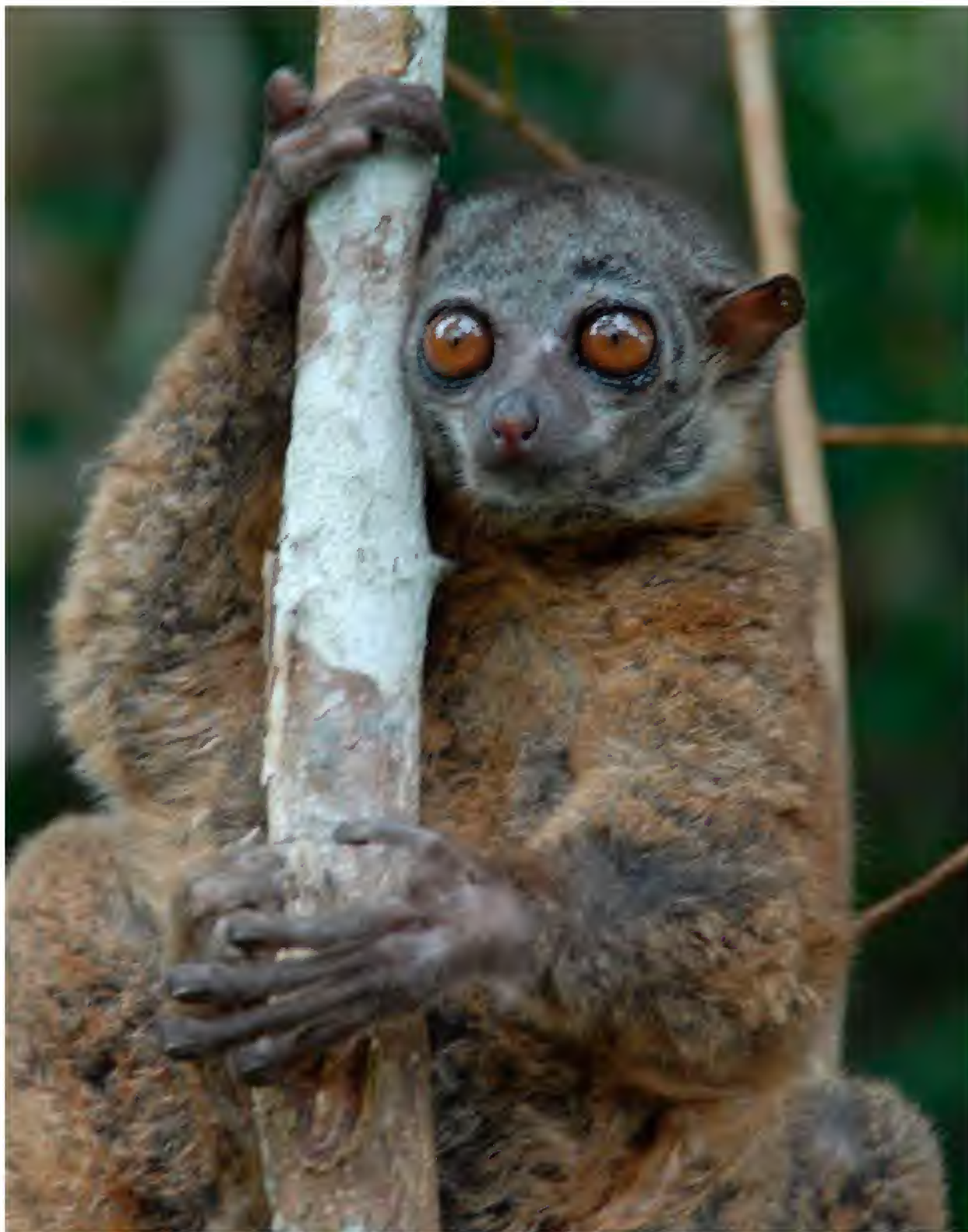


Figure 9. *Lepilemur scottorum*, Scott's sportive lemur. Photo by Edward E. Louis, Jr.

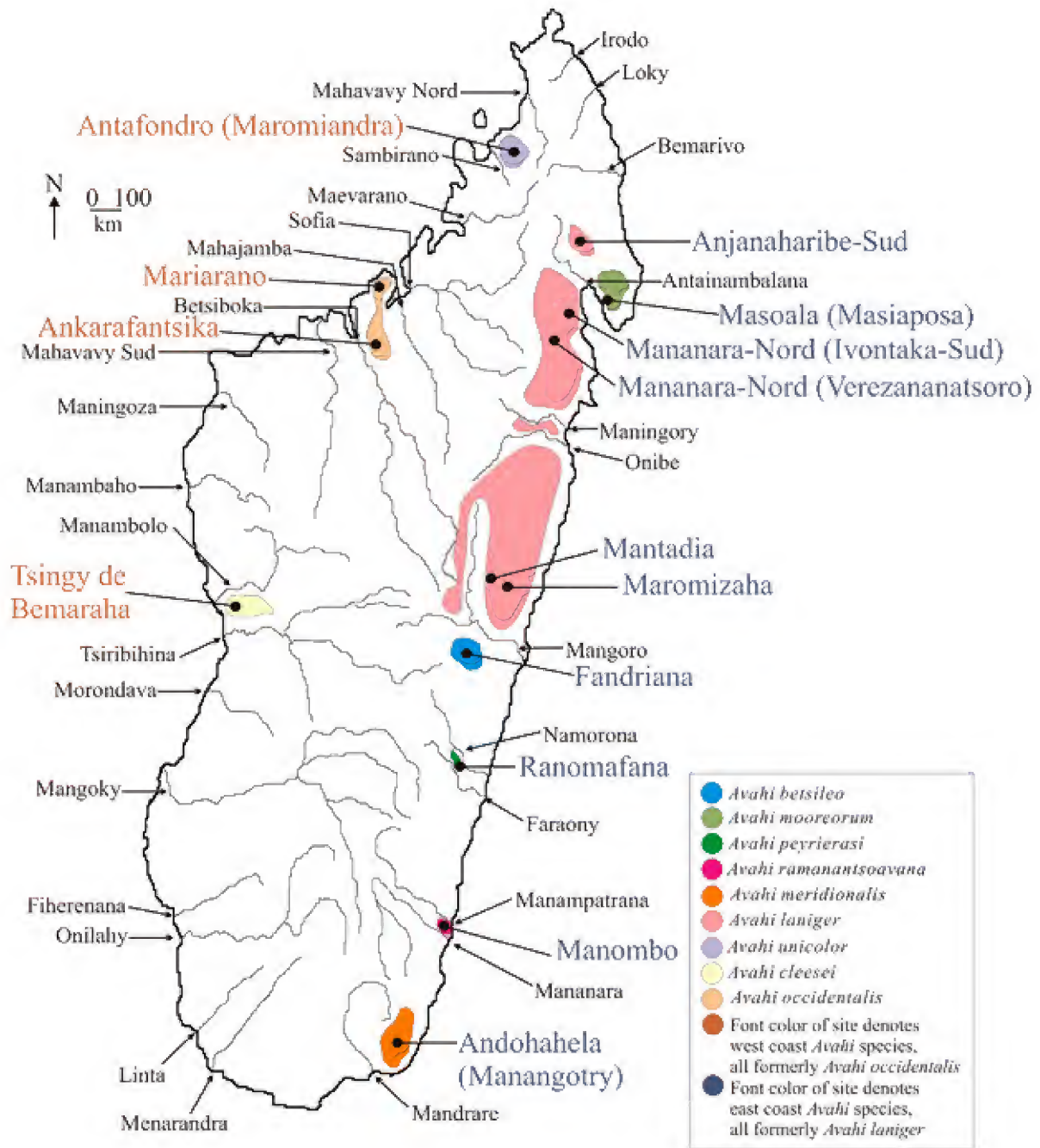


Figure 10. Revised distribution map of the woolly lemurs (genus *Avahi*) of Madagascar.

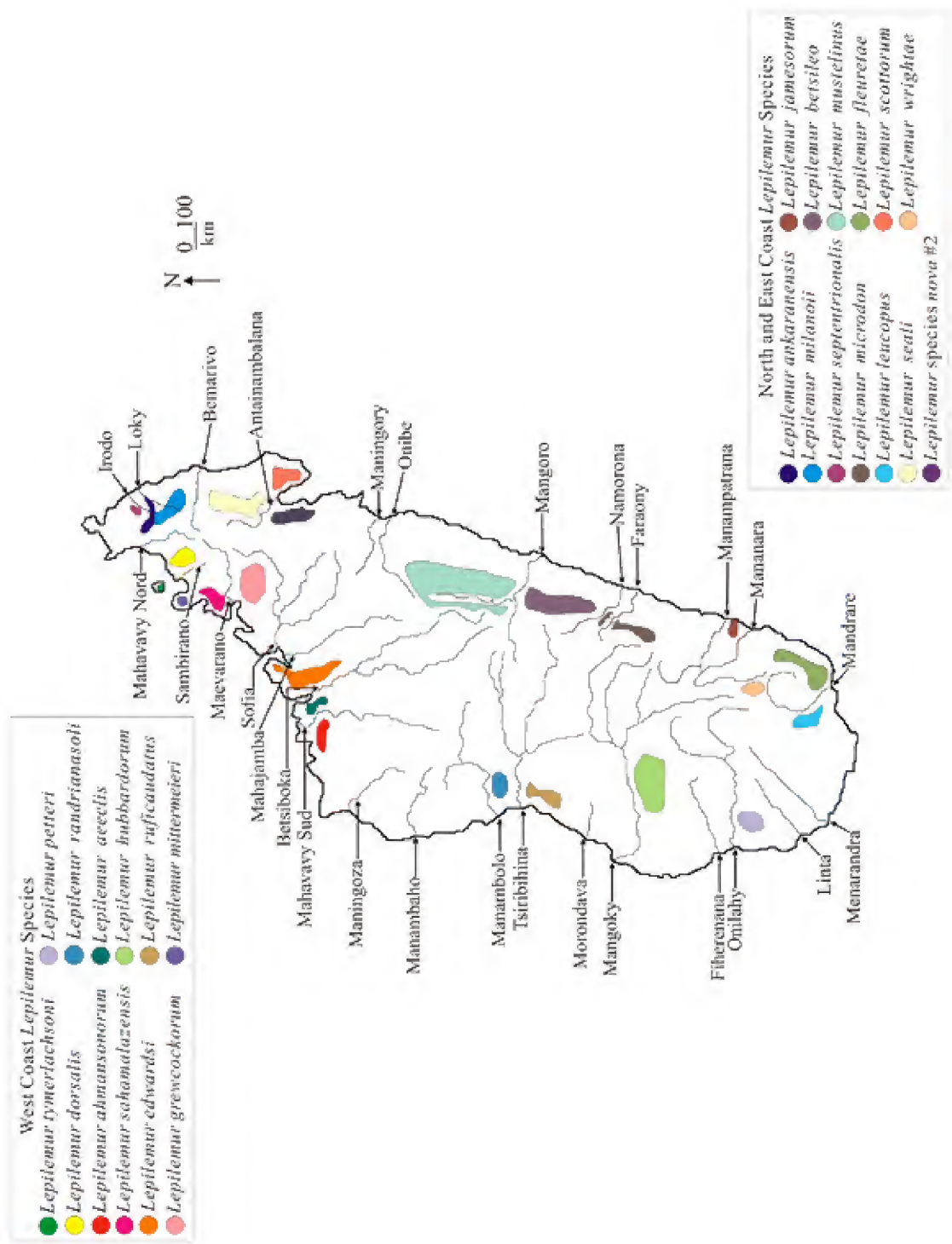


Figure 11. Revised distribution map of the sportive lemurs (genus *Lepilemur*) of Madagascar.

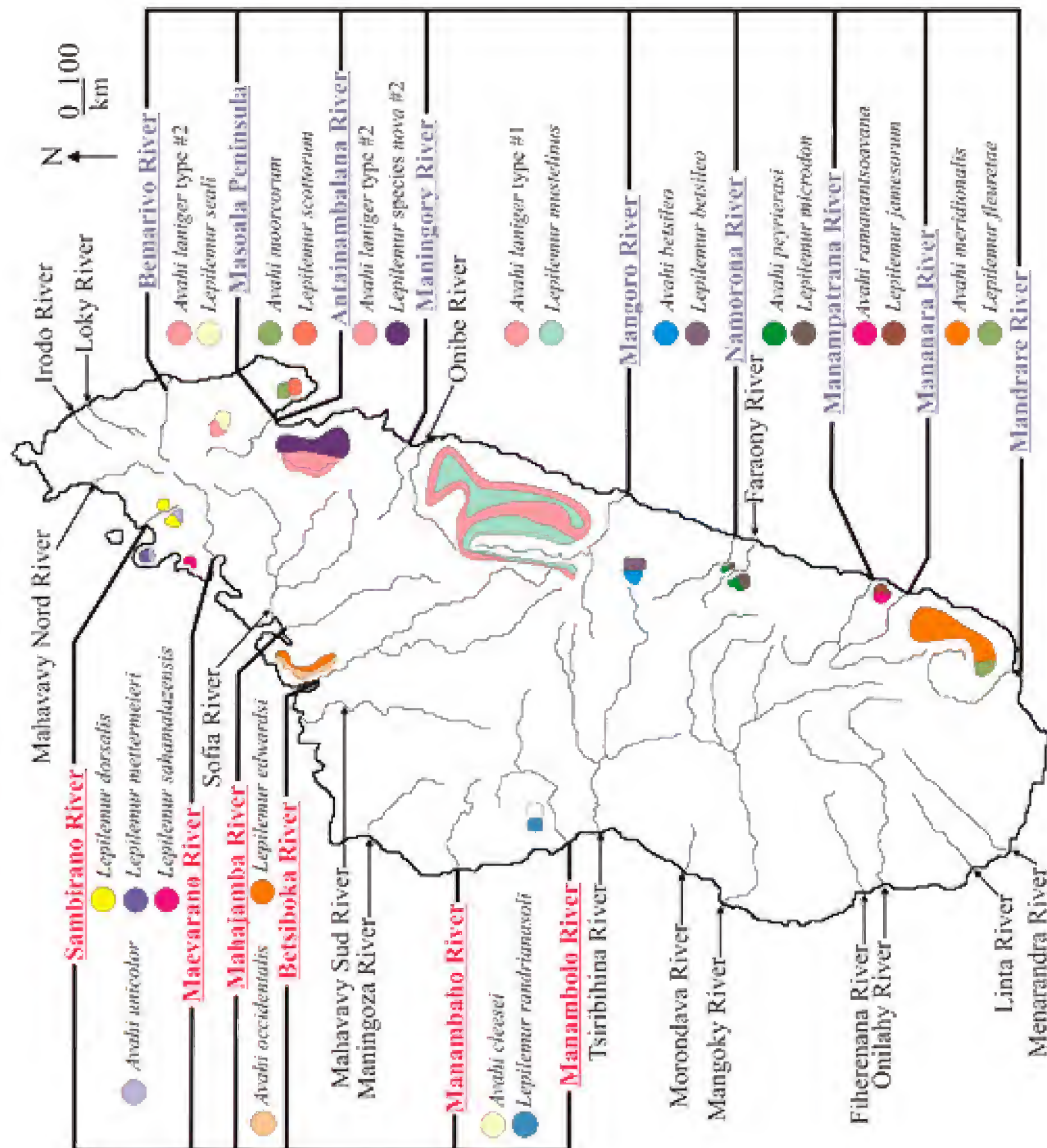


Figure 12. Combined distribution map of the genera *Avahi* and *Lepilemur*. A minimum of one *Avahi* species and one *Lepilemur* species are located between river pairs that act as barriers of the range. Rivers that segregate *Avahi* and *Lepilemur* species on the western half of Madagascar are displayed in red font, and the rivers that segregate *Avahi* and *Lepilemur* species on the eastern half of Madagascar are in blue font.

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The following Appendices I-III to this publication are available online at the indicated website addresses.

APPENDIX I

a. *Avahi* and *Lepilemur* Field Data Appendix (Individual data file for each newly added *Avahi* and *Lepilemur*, including morphometrics, photos, sequence accessions, global position system, microchip data, gender, and location).

<http://10.10.10.3/ccr/genetics/lemur/index.asp?page=ccr/genetics/lemur/AvaLepiMAS.htm>

b. *Avahi* and *Lepilemur* table of individual samples and corresponding information for each sample (bar code number, site, original species designation, current species designation, GenBank accession numbers of sequence data).

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIAvahiLepilemurTable.pdf>

APPENDIX II

- a. Appendix II histograms of means and 95% confidence intervals for significant morphometric parameters found among 11 *Avahi* species or types.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIIAvahiHistogramPartI.pdf>

- b. Appendix II histograms of means and 95% confidence intervals for significant morphometric parameters found among 11 *Avahi* species or types.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIIAvahiHistogramPartII.pdf>

- c. Appendix II morphometric parameter averages and standard deviations among 19 *Lepilemur* species.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIILepiHistogramPartI.pdf>

- d. Appendix II morphometric parameter averages and standard deviations among 19 *Lepilemur* species.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIILepiHistogramPartII.pdf>

- e. Appendix II *Avahi* D-loop fragment haplotypes maximum likelihood phylogram.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIIAvahiDLPHaploML.pdf>

- f. Appendix II *Avahi* PAST fragment haplotypes maximum parsimony phylogram.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIIAvahiPASTHaploMP.pdf>

- g. Appendix II *Avahi* PAST fragment haplotype maximum likelihood phylogram.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIIAvahiPASTHaploML.pdf>

- h. Appendix II *Avahi* D-loop fragment haplotype Bayesian analysis cladogram.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIIAvahiDLPHaploBayesian.pdf>

- i. Appendix II *Avahi* PAST fragment haplotype Bayesian analysis cladogram.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIIAvahiPASTHapBayesian.pdf>

- j. Appendix II *Lepilemur* D-loop fragment haplotype maximum parsimony phylogram.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIILepilemurDLPHaploMP.pdf>

- k. Appendix II *Lepilemur* D-loop fragment haplotype maximum likelihood phylogram.

<http://www.omahazoo.com/ccr/genetics/papers/AppendixIILepilemurDLPHaploML.pdf>

APPENDIX II (CONT.)

- l. Appendix II *Lepilemur* D-loop fragment haplotype Bayesian analysis cladogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*DLPHapBayesian.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>DLPHapBayesian.pdf)

- m. Appendix II *Lepilemur* PAST fragment haplotype maximum likelihood phylogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*PASTHaploML.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>PASTHaploML.pdf)

- n. Appendix II *Lepilemur* PAST fragment haplotype Bayesian analysis cladogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*PASTHaploBayesian.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>PASTHaploBayesian.pdf)

- o. Appendix II *Lepilemur* 12S rRNA fragment haplotype neighbor-joining phylogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*12SrRNAHapNJ.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>12SrRNAHapNJ.pdf)

- p. Appendix II *Lepilemur* 12S rRNA fragment haplotype maximum parsimony phylogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*12SrRNAHapMP.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>12SrRNAHapMP.pdf)

- q. Appendix II *Lepilemur* 12S rRNA fragment haplotype maximum likelihood phylogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*12SrRNAHapML.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>12SrRNAHapML.pdf)

- r. Appendix II *Lepilemur* 12S rRNA fragment haplotype Bayesian analysis cladogram.

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*Lepi*12SHapBayesian.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>Lepi</i>12SHapBayesian.pdf)

- s. Appendix II *Lepilemur* and *Avahi* neighbor-joining phylogram derived from the PAST fragment sequence data from 119 *Avahi* individuals and 216 *Lepilemur* individuals with 25 out-group taxa. Species designated according to distribution in the current literature (Thalmann and Geissmann 2000, 2005; Andriaholinirina et al. 2006; Louis et al. 2006b; Mittermeier et al. 2006; Zaramody et al. 2006; Andriantompohavana et al. 2007).

[http://www.omahazoo.com/ccr/genetics/papers/AppendixII*LepiAvahi*PASTHaploNJ.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixII<i>LepiAvahi</i>PASTHaploNJ.pdf)

- t. Appendix II *Lepilemur* and *Avahi* neighbor-joining phylogram derived from the D-loop fragment sequence data from 119 *Avahi* individuals and 216 *Lepilemur* individuals with 25 out-group taxa. Species designated according to distribution in the current literature.

[http://www.omahazoo.com/ccr/genetics/papers/Appendix*LepiAvahi*DLPHaploNJ.pdf](http://www.omahazoo.com/ccr/genetics/papers/Appendix<i>LepiAvahi</i>DLPHaploNJ.pdf)

- u. Appendix II *Avahi* and *Lepilemur* D-loop and PAST combined fragment haplotype maximum parsimony phylogram.

[http://www.omahazoo.com/ccr/genetics/papers/appendix*AvahiLepilemur*HapMP.pdf](http://www.omahazoo.com/ccr/genetics/papers/appendix<i>AvahiLepilemur</i>HapMP.pdf)

APPENDIX III

- a. Table 1A. Diagnostic nucleotide sites from the D-loop fragment Pairwise Aggregate Analysis (PAA) of *Avahi*.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/AvahiDLPPAA.pdf>

- b. Table 1B. Diagnostic nucleotide sites from the PAST fragment Pairwise Aggregate Analysis (PAA) of *Avahi*.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/AvahiPASTPAA.pdf>

- c. Table 1C. Diagnostic nucleotide sites from the D-loop fragment Pairwise Aggregate Analysis (PAA) for *Lepilemur*.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/LepiDLPPAA.pdf>

- d. Table 1D. Diagnostic nucleotide sites from the 12S rRNA fragment Pairwise Aggregate Analysis (PAA) of *Lepilemur*.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/Lepi12SrRNAPAA.pdf>

- e. Table 1E. Diagnostic nucleotide sites from the PAST fragment Pairwise Aggregate Analysis (PAA) of *Lepilemur*.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/LepiPASTPAA.pdf>

- f. Table 5A. Genetic distance matrix for D-Loop fragment sequence data for *Avahi* species.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/AvahiDLPGDM.pdf>

- g. Table 5B. Genetic distance matrix for PAST fragment sequence data for *Avahi* species.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/AvahiPASTGDM.pdf>

- h. Table 6A. Genetic distance matrix for D-Loop fragment sequence data for *Lepilemur* species.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/LepiDLPGDM.pdf>

- i. Table 6B. Genetic distance matrix for 12S rRNA fragment sequence data for *Lepilemur* species.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/Lepi12SrRNAGDM.pdf>

- j. Table 6C. Genetic distance matrix for PAST fragment sequence data for *Lepilemur* species.

<http://www.omahazoo.com/ccr/genetics/papers/appendixIII/LepiPASTGDM.pdf>

APPENDIX III (CONT.)

k. Appendix III Haplotype Table I (Summary of designated haplotypes for the genus *Avahi* from all localities for D-loop and PAST fragments).

[http://www.omahazoo.com/ccr/genetics/papers/AppendixIII*Avahi*HaplotypeTableI.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixIII%20AvahiHaplotypeTableI.pdf)

l. Appendix III Haplotype Table II (Summary of designated haplotypes for the genus *Lepilemur* from all localities for D-loop, 12S rRNA, and PAST fragments).

[http://www.omahazoo.com/ccr/genetics/papers/AppendixIII*Lepilemur*HapTableII.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixIII%20LepilemurHapTableII.pdf)

m. Appendix III Haplotype Table III (Summary of designated haplotypes for the genus *Lepilemur* from localities for D-loop, ND3 and ND4L fragments from Craul et al. (2007).

[http://www.omahazoo.com/ccr/genetics/papers/AppendixIII*Lepilemur*CraulTableIII.pdf](http://www.omahazoo.com/ccr/genetics/papers/AppendixIII%20LepilemurCraulTableIII.pdf)

